STUDIES IN THE BIOLOGY AND REPRODUCTIVE CHARACTERISTICS OF *PSEUDOMUGIL SIGNIFER*.

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy.

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A

B

Photographs of the southern male (A) and female (B) *Pseudomugil signifer*. Photographs courtesy of Mr R. H. Kuiter.

CERTIFICATE.

I certify that this thesis has not already been submitted for any degree and is not being submitted as part of candidature for any other degree.

I also certify that this thesis has been written by me and that any help that I have received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

Production Note:
Signature removed prior to publication.

Effie Howe

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"The fish in the creek said nothing. Fish never do. Few people know what fish think about injustice or anything else."

Catwings. Ursula Le Guin (1988)

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ABSTRACT.

The aims of this study were firstly to observe the breeding behaviour and embryology, and then to identify factors affecting the reproductive biology of the Australian native pseudomugilid *Pseudomugil signifer* (Pacific blue-eye) and the impact upon it of the presence of the exotic species *Gambusia holbrooki* (eastern gambusia). Six species of the genus *Pseudomugil*, and the related *Scaturiginichthys vermeilipinnis*, are found on the Australian continent. The normal breeding behaviour, egg surface morphology and embryology of four species of *Pseudomugil* (*P. signifer*, *P. gertrudae*, *P. tenellus* and *P. mellis*) were first investigated, using aquarium and microscopic (light and S.E.M.) studies. The four species were divided into two groups: *P. signifer* and *P. mellis*; and *P. tenellus* and *P. gertrudae*. The study provided further evidence for the view that the embryology of the genus *Pseudomugil* differs markedly from that of members of the family Melanotaeniidae, with which the pseudomugilids have previously been grouped.

The seasonal pattern of gonadal function in *P. signifer*, both in the field and in aquariums, was then investigated for populations of *P. signifer* from the Sydney region. It was found that *P. signifer* bred over the spring and summer months, commencing breeding as the temperature and daylength increased, and declining in breeding activity as daylength and temperature declined. There was no substantial difference in the pattern of reproductive activity between wild and captive stocks of *P. signifer* in the populations used.

The impact of the presence of the introduced *G. holbrooki* on *P. signifer* was then examined, first in open-air tank experiments, and then in the field. In the tank experiments the exotic species profoundly affected the breeding of the native species. When *G. holbrooki* were in the tanks *P. signifer* did not gain weight or grow in total length (except for females given supplementary feed); ovarian weight and fecundity was greatly reduced and the ovaries were morphologically undeveloped. No eggs from *P. signifer* were observed in tanks which also housed *G. holbrooki*. *G. holbrooki* were observed to actively hunt and eat young *P. signifer* and to nip the caudal fins of adult *P. signifer*. The results indicate clearly, that at least in a captive situation, the presence of the exotic species has a very deleterious effect on breeding and hence possible survival, of a native population.

A pilot study conducted at the same time as the harvest of the second tank study did not reveal such drastic consequences. However, even in the less confined field situation, some evidence of an interrelation between water quality, numbers of *P. signifer* and numbers of *G. holbrooki* were seen in one disturbed site (Homebush Bay). These findings suggest that a newly designed field experiment based on data collected from the power analysis of the pilot study could clarify whether *G. holbrooki* adversely affects *P. signifer* in the wild.

The information gained from these studies can be used in the management of *P. signifer* in the wild, and serve as a model of the possible effects upon other native species.

Chapter 1.

General Introduction.

Studies in the biology and reproductive characteristics of *Pseudomugil signifer*.

1.1 Context of project:- Australian freshwater fish fauna and the aims of the project.

Australia has approximately 195 endemic freshwater fish species and subspecies which have been formally described and a further 20 recognised but undescribed species or subspecies (Wager and Jackson, 1993). This is a small number when compared with that in other countries (Merrick and Schmida, 1984). In comparison, it has been estimated that there are 2,500 species of fish in the Amazon Basin (Petrovický, 1988). The small number of species in Australia has been attributed to the isolation of the continent for fifty million years and "the geologic and climatic condition" during this period (Merrick and Schmida, 1984). All except four species (Queensland lung fish (*Neoceratodus forsteri*), northern and southern saratogas (*Scleropages jardini* and *Scleropages leichardti*) and salamander fish (*Lepidogalaxias salamandroides*)) are considered to be derived relatively recently from marine ancestors and are not highly specialised (Wager and Jackson, 1993).

One of the most significant factors affecting the distribution of Australian freshwater fish species is geographic barriers, in particular the drainage division boundaries. These are of relatively recent origin in their present form (Merrick and Schmida, 1984) (Fig. 1.1.).

Species diversity and abundance have been compromised by changes in the freshwater aquatic environment due to human activity. Changes in Australian freshwater streams resulting from human activities can be divided into three categories:- physical, chemical and biological (Merrick and Schmida, 1984). The biological changes are most directly concerned with the aims of this study. They are associated with the introduction of fish and plants and disease organisms (enhanced by the diversion and connection of waters previously isolated).

Research and management of freshwater fish in Australia generally concentrates on commercially important exotic species.

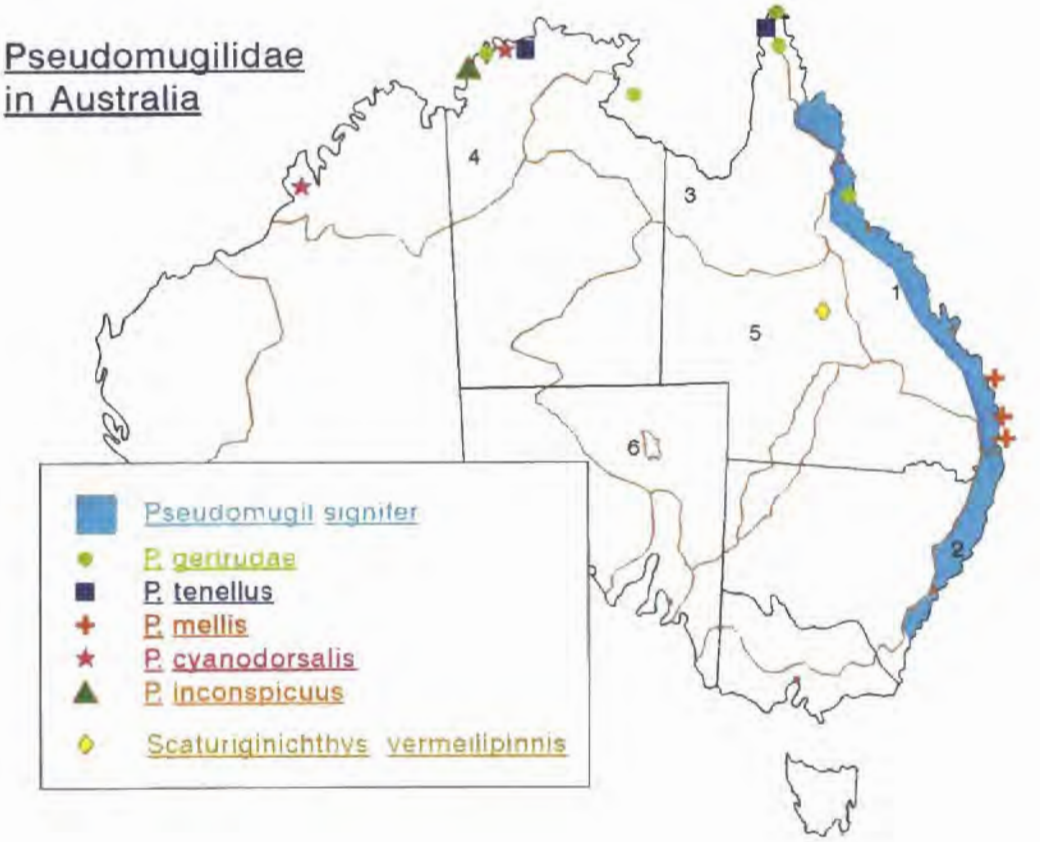


Figure 1.1 Drainage division boundaries on the Australian continent (Merrick and Schmida, 1984) and distribution of *Pseudomugil* species (Allen, 1989 and Ivantsoff *et al.*, 1991). 1. North East Coastal Division 2. South East Coastal Division 3. Gulf of Carpentaria Division. 4. Timor Sea Division. 5. Lake Eyre Division. 6. Lake Eyre.

A search of the Aquatic Science and Fisheries Abstracts (ASFA) database for the period 1978-1994 on Australian freshwater fish yielded 391 entries, of these 187 referred to salmonids or cyprinids (both introduced). It is only in recent times that there has been interest in commercially important native freshwater fish (Lake, 1967a and 1967b; Mackay, 1973; Rimmer, 1985; Harris, 1986 and Harris, 1988). In the ASFA search referred to above the native species, barramundi (*Lates calcifer*), was referred to in 129 instances, and the genus *Macquaria*, also commercially important, was next commonly mentioned (51 instances). Little attention has been paid to management of smaller native freshwater fish stocks with the exception of an action plan for Australian freshwater fishes (Wager and Jackson, 1993). By contrast with the large commercially important species there were 13 citations to *Pseudomugil* species in the ASFA search. Smaller fish species are of vital importance from both a conservation aspect and as a food source for the larger fish. They are an important part of the aquatic ecosystem and it is important to understand their biology in terms of maintaining species diversity and quality of the aquatic environment. Before management studies can be performed and management policies devised, it is essential to know the basic biology (including reproduction and embryology) and ecology of the fish. For example, the inland species golden perch *Macquaria ambigua*, requires a water level rise for spawning (Lake, 1967a and 1967b). Damming rivers and thereby changing flow rates can be detrimental to this species.

The aim of the current project has been to describe the biology of an abundant and widespread, noncommercial, small, nonangling, freshwater/estuarine fish and to describe some of the factors that control its reproduction and growth under both laboratory and field conditions. The project has focussed on egg morphology and breeding behaviour of the blue-eye *Pseudomugil signifer* and three related species, the breeding cycle of *P. signifer*, being taken as a representative of the genus, in relation to environmental factors. Laboratory and field studies were also carried out on the effects on fecundity and growth of *P. signifer* of the presence of the exotic species, *Gambusia holbrooki*.

1.2 Review of factors affecting growth and survival in *Pseudomugil.*

The review looks at biological factors that affect the success of fish generally and this genus particularly. Success is defined here as maintenance of, or increase in numbers of fish, and growth of individuals. Following this review the basis for the current study is presented and explained.

1.2.1 Water quality parameters.

Changes in pH, water temperature, dissolved oxygen levels, suspended sediments and salinity are known to have subtle but crucial effects on survival, growth and reproduction in fish populations (Koehn and O'Connor, 1990). In addition photoperiod and temperature are considered to be the most important environmental cues for gametogenesis in temperate climate fishes (Lam, 1983). The balance between these two apparently varies between species, Hubbs (1971) suggesting that light rather than temperature was the main controller of reproductive seasonality in *Gambusia* sp., since the beginning and end of reproduction occurred at the same time of year, independently of either thermally stable or cycling environments.

1.2.1.1 pH

P. signifer has been found over a pH range of 5.5-7.8 (Allen and Cross, 1982). pH also interacts with other parameters such as the concentration of free ammonia in water (Piper *et al.*, 1982). Although individual species have their own pH preferences, broadly acceptable levels for fish culture are considered to be between 6.5-9.0 (Cook, 1986). Clearly each species has its own range of tolerance of pH, the exact limits of which may in turn be affected by other factors.

1.2.1.2 Temperature.

Fish are poikilotherms having a variable body temperature, and therefore are very dependent on the surrounding water temperature. If the water temperature is outside the optimal range for a particular species, stress, change in growth rate, decreased feeding, and eventual death may occur (Hart, 1974; Koehn and O'Connor, 1990). On the other hand, a seasonal change in the water temperature, within the optimal

range, can be the signal for migration or spawning (Nikolsky, 1963; Lasker, 1974). The functioning of the fish's reproductive system is influenced by temperature (Cook and Peter, 1980), however few studies have shown that temperature directly stimulates endocrine events leading to ovulation (Stacey, 1984). Increased water temperature was found to induce ovulation in goldfish (Yamamato *et al.*, 1966), but on the other hand goldfish have also been found to ovulate with the introduction of vegetation, without a temperature increase (Stacey *et al.*, 1979).

A change in the water temperature can be caused by industrial processes. For example, water used as a cooling agent in the generation of electric power may be released at a higher temperature than the surrounding waters. It is for this reason that temperature monitoring around such installations is necessary for the well being of fish life (Alabaster and Lloyd, 1982). Temperature also affects other parameters such as dissolved oxygen (Hart, 1974) and the concentration of pH and hence free ammonia (Alabaster and Lloyd, 1982).

1.2.1.3 Dissolved oxygen.

Dissolved oxygen concentration is considered the most important chemical component of natural waters (McLarney, 1987). Acceptable continuous exposure levels in water for fish culture should be greater than 6 mg/l (up to 100% saturation) (Langdon, 1988). It has been found that dissolved oxygen concentrations should not be lower than 5 mg/l for stages of high sensitivity during fish development, and for short periods, of less than 24 hours, a minimum of 4 mg/l can be tolerated (Hart, 1974). Dissolved oxygen concentration is affected by other environmental parameters, in particular, temperature and salinity (Glazebrook *et al.*, 1988). More active fish require more oxygen, especially with increasing water temperature. However the dissolved oxygen at saturation declines with increasing temperature (McLarney, 1987).

1.2.1.4 Biological oxygen demand (BOD).

Dissolved oxygen is essential to most aquatic life. It is consumed by the metabolic activities of water column and benthic organisms, including micro-organisms. The oxygen-consuming capacity of a water sample due to microbial respiration during

biodegradation is known as biological oxygen demand or BOD (Langdon, 1988). The BOD is a standardised test to assess the potential oxygen demand by biological material in the water.

1.2.1.5 Ammonia.

Ammonia is a product of the breakdown of protein by aquatic organisms. Ninety eight percent of ammonia is excreted via the gills in fish (Maetz and Evans, 1972). Generally this is achieved by passive transport as NH_3, but sometimes it is excreted as NH_4^+ (in exchange for Na^+ ions) (Smart, 1981). In large volumes of water ammonia is diluted and broken down but in crowded situations or in small volumes of water it can become toxic to fish (Ferguson, 1988). Ammonia is thought to decrease the ability of haemoglobin to combine with oxygen resulting in the suffocation of the fish (Hart, 1974). The majority of research into tolerances of fish species to ammonia has been conducted on European species, in particular salmonids which appear to be more sensitive (Reddacliff, 1985). There is wide variation in the tolerance of fish species for ammonia. On analysis of different fish species an ammonia concentration of 0.2-2.0 mg/l NH_3 was considered to be acutely lethal (trout were most sensitive, common carp the most resistant) (Alabaster and Lloyd, 1982). Un-ionised ammonia concentrations of more than 0.0125 mg/l resulted in a decline in trout quality evidenced by reduction in growth rate, damage to gill, kidney, and liver tissues (Piper *et al.*, 1982). However, salmonids have been recognised as being highly sensitive to water quality (Reddacliff, 1985). Reduced growth rate and gill damage occurred in channel catfish exposed to 0.12 mg/l or greater of un-ionised ammonia (Piper *et al.*, 1982). It should be noted that not all species have been assessed for their tolerance to un-ionised ammonia and reported acute toxicity varies widely between species (Reddacliff, 1985). Salmonids show severe distress at un-ionised ammonia concentrations of 0.3 mg/l, while at the other extreme, ammonia is reportedly used as a fertiliser, and for algal control, in carp and tilapia ponds in Israel at levels of more than 10 mg/l without apparent deleterious effects on the fish (Reddacliff, 1985). This exceeds the acute tolerance reported for common carp by Alabaster and Lloyd (1982) by 5-fold.

Since no data is available on levels of ammonia that *P. signifer* will tolerate, it

can only be deduced that they would be more tolerant than salmonids since they can be found in polluted water.

Tolerance of fish species of dissolved ammonia has been presented in terms of either total ammonia or un-ionised ammonia, however the latter is more informative. When ammonia gas dissolves in water, some combines with the water to form ammonium ions which are relatively non-toxic to fish. The rest is present as un-ionised ammonia (NH_3) (Piper *et al.*, 1982). The analytical test performed on the water samples yields total ammonia concentrations, as standard analytical methods cannot distinguish the two forms (Piper, *et al.*, 1982). The amount of free ammonia varies with pH and temperature. In acid conditions, most ammonia occurs as the relatively non-toxic ammonium ion. Thus any test for ammonia in water must be accompanied by pH and temperature readings (Environmental Protection Agency, 1976; Piper *et al.*, 1982). Wuhrmann and Woker (1948) in Alabaster and Lloyd (1982) showed that only the un-ionised molecule was toxic, the ammonium ion having little or no toxic effect.

The percentage of un-ionised ammonia present in an ammonia solution is given as

$$\% \text{ un-ionised ammonia} = 100/(1+\text{antilog}\ (pK_a\text{-}pH)),$$

where pK_a is the negative logarithm of the dissociation constant for ammonia.
The reaction of ammonia excreted into water by fish takes the following form (from Piper *et al.*, 1982):

$$NH_3 + H_2O \leftrightarrows NH_4OH \leftrightarrows NH_4^+ + OH^-$$

$\Uparrow$ $\Uparrow$ $\Uparrow$

un-ionised toxic form pH dependent ionised non-toxic form

However Cook (1986) suggests that 0.1 mg/l NH_3 is acceptable for aquaculture in Australia. Langdon (1988) on the other hand suggests a level of 0.02 mg/l un-ionised ammonia as acceptable for continuous fish culture. Acceptable concentrations of ammonia appear to differ between studies. Very little research on optimum water conditions for Australian fish has been performed. Since different species vary in their tolerance to ammonia (Alabaster and Lloyd, 1982), it would be of great interest to have more details on ammonia tolerance levels for different Australian native fish species.

1.2.1.6 Nitrate.

Nitrogen is an essential element and nitrate the commonest form in animal uptake. In aquatic systems nitrate is produced by the complete oxidation of ammonia (Ferguson, 1988).

There are a number of major anthropogenic, non-point sources, including (in order of overall magnitude) agricultural runoff from fertiliser application, sewage, and stormwater runoff (Connell, 1993). Eutrophication of water bodies due to increased levels of nutrients, including nitrates, leads to increased plant growth. Detrimental effects on fish are due to two causes, other than direct toxicity. Firstly decomposition of large masses of plant material leads to low dissolved oxygen concentrations. Secondly, blooms of cyanobacteria may release toxic substances into the water (Garmen and Sutherland, 1983 and Connell, 1993).

Less than 1 mg/l is considered acceptable for continuous exposure in fish culture (Langdon, 1988). Carp should not be subjected to more than 80 mg/l and rainbow trout to not more than 20 mg/l nitrate (Svobodova *et al.*, 1993).

1.2.1.7 Hardness.

Total hardness is defined as the sum of the calcium and magnesium concentrations (APHA, 1992). A total hardness of between 20-200 mg $CaCO_3$/l is considered acceptable for continuous exposure in fish culture (Langdon, 1988).

1.2.1.8 Suspended sediment.

Water contains both organic and inorganic suspended solids. Organic particles are thought to be more hazardous to fish than the inorganic particles, although the reasons for this are unclear (Ferguson, 1988). It has been suggested that increased turbidity reduces fish growth, affects feeding, increases stress and reduces photosynthesis leading to low plant growth (Koehn and O'Connor, 1990). More importantly these authors point to the extreme effects if the sediment settles, reducing the number of spawning sites and food sources. Data are not available on acceptable levels of suspended sediment for Australian fisheries but it has been found that the level of non-filterable finely-divided solids should be less than 80 mg/l for European freshwater fish (Hart, 1974). Alabaster and Lloyd (1982)

tentatively suggested that concentrations of suspended solids less than 25 mg/l are not harmful, 25-80 mg/l are moderately good, 80-400 mg/l are moderately bad, and above 400 mg/l would not be considered good for fisheries. However, none of their data were from Australian fisheries.

In the current study, in both the laboratory and field investigations, pH, temperature, dissolved oxygen, ammonia, nitrate and hardness were measured and their possible influence on the results was considered.

1.2.2 The genus *Pseudomugil.*

1.2.2.1 Taxonomy.

Pseudomugil is a genus of atheriniform teleosts (Jamieson, 1991) which are considered to be the most primitive of the rainbowfishes (Allen and Cross, 1982), although no known fossil records exist of rainbowfishes. According to Allen (1980) rainbowfish represent an offshoot of the atherinid evolutionary line. *Pseudomugil* is derived from Latin, meaning "false mullet".

Opinions have differed as to the taxonomic position of the genus. Initially, the genus was placed in the family Pseudomugilidae (Kner, 1865), a classification accepted by Munro (1958) and Grant (1978). On the other hand, Günther (1867 a and b), Shipway (1947) and Lake (1971) included them in the family Atherinidae. These early classifications did not involve comprehensive taxonomic investigations of the genus. More recently the genus was grouped with the Melanotaeniidae (Allen, 1980), on the basis of two shared characteristics :- the jaw structure and the unique modification of the pelvic fins. Saeed *et al.*, (1989) performed an extensive osteological study on *Pseudomugil* species and concluded that they should be placed in their own family, Pseudomugilidae, on the basis of the following apomorphic characters: "absence of mesethmoid; presence of only one anterior infraorbital; articular as high as dentary and always lower than dentary in other members of Atherinoidea". This classification is satisfactory since it is based on a recent, highly systematic and comprehensive study of the osteology of the genus using modern methods.

P. signifer was first described from a Sydney specimen by Kner (1865). There has been controversy as to whether the northern and southern populations of *P. signifer*

found along the east coast of Australia belong to the same species. Günther (1867a), McCulloch and Whitley (1925) and Hadfield, *et al.* (1979) subscribed to the hypothesis that there was only one species; however Günther (1867b), Jordan and Hubbs (1919), Whitley (1932) and Munro (1958) took the view that there were two species within this range.

Günther (1867a) described a species from Cape York, Queensland, as *Atherina signata* but later in the same year (1867b) he identified it as being the same as *P. signifer* (Günther, 1867b). Macleay (1881) described a new species from the Bremer River near Brisbane as *Atherinosoma jamesonii*. In 1919, Jordan and Hubbs again subscribed to the opinion that the Cape York species *P. signatus* was distinct from the southern population. In 1925 McCulloch and Whitley placed *P. signatus, A. jamesoni* and *P. signifer* together as *P. signifer*. In 1932, Whitley redivided the species into the northern (*P. signatus)* and the southern (*P. signifer*). In 1935, Whitley proposed that specimens from the Low Isles in Queensland represented a new sub-species, *P. signatus affinis,* as it had a higher dorsal ray count than the holotype. In 1958, Whitley, using the diagnostic characters of colour and body markings, proposed that *P. signifer's* distribution was in NSW and southern Queensland, *P. signatus* in northern Queensland and *P. signatus affinis* in the northern Queensland islands. Munro (1958) was in agreement with Whitley (1958), basing his separation of the species on orbit length, snout length and the degree of lateral stripe. However, the most recent study addressing this problem, which was a comprehensive electrophoretic and morphological study from 14 localities along the east coast of Australia, identified them as one species (Hadfield, *et al.*, 1979). These authors suggested the differences observed by Munro (1958) to be clinal variations.

1.2.2.2 Distribution.

Several major geologic events influenced the distribution of Australian freshwater fish (Merrick and Schmida, 1984):- the separation of Australia from Gondwanaland; the relative proximity of the Indian sub-continent and Australia until 50 million years ago; the late Miocene marine regression, when many Pacific shallow shelf areas were shallow lakes; the formation of New Guinea and the repeated emergence and submergence of extensive land bridges on the Sahul Shelf.

The genus *Pseudomugil* comprises 12 species (Table 1.1), six of which have been found in Australian waters, namely *P. signifer* , *P. mellis*, *P. tenellus*, *P. gertrudae*, *P. cyanodorsalis*, *P. inconspicuus*. *Scaturiginichthys vermeilipinnis* is a recently described pseudomugilid from western Queensland (Saeed *et al.*, 1989).

Members of the family are found in eastern, northern and north-western Australia, Papua New Guinea and several adjacent islands (Allen and Cross 1982; Allen and Sarti 1983). They are found in a total of five Australian drainage divisions (Timor Sea division, Carpentaria division, North East Coast division and South East Coast division and the Lake Eyre division). *P. signifer* is the most widely distributed species of *Pseudomugil* and is abundant along the east coast of Australia (the North East Coast and South East Coast drainage divisions), but is not found far inland (Fig. 1.1). It is relatively tolerant of variations in salinity, pH and temperature. *P. signifer* is not found in the Northern Territory where four other species are found (*P. tenellus, P. cyandorsalis*, *P. inconspicuus* and *P. gertrudae*). A sixth species, *P. mellis* is found in a limited area of south eastern Queensland. It is more restricted in its habitat requirements and has been classed as a "vulnerable" species in recent analyses of the rare and endangered freshwater fishes of Australia (Wager and Jackson, 1993 and Jackson, 1994).

In addition, a new genus and species of the family Pseudomugilidae the redfinned blue-eye, *Scaturiginichthys vermeilipinnis*, has been discovered from inland aquifers of the Lake Eyre Drainage division (Ivantsoff *et al.* 1991). It is also considered "endangered" (Wager and Jackson, 1993 and Jackson, 1994).

1.2.2.3 Biology.

Description.

Members of the genus *Pseudomugil* are very small (30-70mm total length). Most species are sexually dimorphic with the males having more colourful fins than the females. Mature specimens of Australian species can be easily identified by eye.

The eyes of *Pseudomugil* species are distinguished by an iridescent blue appearance and hence they have commonly been referred to as "blue-eyes". Lanzing and Wright (1981) published an account of the fine structure of the chromatophores and other non-sensory components of the eye of *P. signifer*.

Table 1.1.

Species of Pseudomugilidae occurring in Australia and Papua New Guinea.

Described *Pseudomugil* species.	Described by
1. *P. signifer* * ●	Kner, 1865
2. *P. mellis* * ●	Allen and Ivantsoff, 1982
3. *P. tenellus* * ●	Taylor, 1964
4. *P. gertrudae* * ●	Weber, 1911
5. *P. inconspicuus* *	Roberts, 1978
6. *P. cyandorsalis* *	Allen and Sarti, 1983
7. *P. paludicola*	Allen and Moore, 1981
8. *P. novaeguineae*	Weber, 1908
9. *P. paskai*	Allen and Ivantsoff, 1986
10. *P. helodes*	Ivantsoff and Allen, 1984
11. *P. majusculus*	Ivantsoff and Allen, 1984
12. *P. reticulatus*	Allen and Ivantsoff, 1986
13. *S. vermeilipinnis* *	Ivantsoff, Unmack, Saeed & Crowley, 1991

* Found in Australia.
● Species used in this project.

Comparisons were made with the composition of the eye of G. *holbrooki*. It was suggested that the blue iris of *Pseudomugil* was due to the manner of organisation of the irideal type *B* iridophores.

Four species are included in the present investigation:-

P. signifer - was formally described by Kner in 1865. The name "*signifer*" means marked or signed. They are the largest and best known species of *Pseudomugil,* the males reaching a length of 70 mm. They lack black spots on the fins or body, unlike *P. gertrudae*. There is clinal variation in body shape, fin shape and colour (Allen and Cross, 1982). They are sexually dimorphic.

P. mellis - was formally described by Allen and Ivantsoff in 1982. The name "*mellis*" comes from the Latin and refers to the male's honey colouration. They

reach a maximum length of 30 mm, and they are sexually dimorphic. The males have black and white banding on the dorsal, caudal and anal fins. The males have similar fin colouration to *P. signifer* and were initially thought to be a geographic variant of this species. However, Allen and Ivantsoff (1982) found *P. mellis* to differ in head shape and pore pattern, dentition and colouration of adult males. It is of interest to this project that *P. mellis* is considered endangered and to be under threat by *G. holbrooki.*

P. tenellus - was formally described by Taylor in 1964. The name "*tenellus*" refers to its small delicate appearance (Taylor, 1964). It has no spots on the fins or body. The maximum length recorded is 50mm. Body colouration is yellowish-brown. The base of the second dorsal and anal fin is dark and has clear to yellowish outer margins (Allen and Cross, 1982). The species is not obviously sexually dimorphic.

P. gertrudae - was formally described by Weber in 1911. It is commonly called the spotted blue eye. Dark margins of scales form black spots at intersections producing horizontal rows of spots on the body (Munro, 1967). The fin area is also spotted. It is one of the smaller species growing to about 30mm standard length. There are three colour variations depending on the drainage division in which they occur (Merrick and Schmida, 1984). They have strong sexual dimorphism. Mature males have longer 2nd dorsal fins than females. The colour of the pectoral fins can be related to the drainage area from which they come. They are coloured bright orange when caught in Arnhem Land or white from Cairns (Merrick and Schmida, 1984).

Other *Pseudomugil* species found in Australia (not included in this project). -
P. cyanodorsalis was formally described by Allen and Sarti in 1983. It is the only *Pseudomugil* species that has been found in Western Australia and is also found in the Northern Territory. *P. inconspicuus* was described by Roberts in 1978. It was initially thought to come only from the lower Fly River in south western Papua New Guinea, but has recently been found in mangrove swamps near Darwin in Australia.

Scaturiginichthys vermeilipinnis (the red finned blue-eye) was described by Ivantsoff *et al.* (1991). It is of considerable interest that this newly discovered

species was found in inland waters of the Lake Eyre drainage system, sharing its habitat with the mosquitofish *Gambusia holbrooki*. It is considered endangered for this reason, and because its habitat, western Queensland mound springs, is under pressure due to cattle grazing and trampling.

Reproduction.

Information on breeding is derived almost entirely from captive studies. *P. signifer* is a hardy fish that has been kept by aquarists for many years; successful maintenance techniques have been published (Merrick and Schmida, 1984; Semple, 1986; Leggett and Merrick, 1987). *P. signifer* are oviparous, producing 4-9 eggs each day for up to a week at a time (Leggett and Merrick, 1987; Allen and Cross, 1982). They spawn on the roots of floating plants, the eggs attaching to plants by sticky threads (Merrick and Schmida, 1984; Leggett and Merrick, 1987). Breeding in aquariums has been achieved and larval development has been studied by Semple (1986). The eggs take between 18-21 days to hatch at a temperature of 22-24°C (Merrick and Schmida, 1984). *P. signifer* bred in aquariums take 6 months to reach sexual maturity (Allen and Cross, 1982).

Less detail is available on other Australian species of *Pseudomugil*. *P. mellis* have been found to spawn 6-15 eggs on roots of floating plant material in a day for several days at a temperature of 25-30°C (Leggett and Merrick, 1987). However, a more recent study by Semple (1991) found that spawning commenced when the temperature reached 28°C with the release of 42-125 eggs within 9 days, followed by 4-9 days rest. A single pair could produce up to 15 eggs in a day, the eggs commenced to hatch within 5 days at a temperature of 26-30°C (Semple, 1991). Only *P. tenellus* have been examined in the field. Bishop *et al.*, (1995) examined the gonads of a sample of *P. tenellus* (35 males, 69 females and 84 juveniles). *P. tenellus* were not captured in breeding condition and no place where spawning occurred was identified. Under aquarium conditions at a temperature of 25-30°C *P. tenellus* have been found to spawn small numbers of eggs that take 4-6 days to hatch (Leggett and Merrick, 1987). *P. gertrudae* have been observed to spawn in groups in aquariums (Merrick and Schmida, 1984), and the eggs take up to one and a half weeks to hatch at 22-25°C (Allen and Cross, 1982) or 27°C (Semple, 1985a). They

have been found to spawn several times a day under aquarium conditions, each female producing up to ten eggs in a day.

1.2.2.4 Ecology.

P. signifer are generally found in an estuarine environment. They prefer a habitat with plenty of aquatic vegetation in flowing water (McDowall, 1980). *P. mellis*, *P. gertrudae* and *P. tenellus*, like *P. signifer*, are all found in water with dense vegetation. However, unlike *P. signifer* they are generally found in a freshwater habitat with little or no flow (Allen and Cross, 1982; Merrick and Schmida, 1984).

Both current and salinity affect life in an estuary (Smith, 1966). For example under the winter-rainfall pattern seen in south-eastern Australia, salinity is at its highest during summer and during periods of drought and lowest in winter and spring when rivers and streams bring down large volumes of freshwater. Changes in salinity can be very dramatic after prolonged rain periods. Temperatures in estuaries also can vary greatly both diurnally and seasonally. Smith (1966) believed that the mixing of different salinities and temperatures acts as a nutrient trap thus improving the food supply in the estuary. Therefore *P. signifer* unlike other species of *Pseudomugil* have to adapt to these conditions.

Bishop *et al.* (1995) reported information on the abundance, biomass, habitat preferences, reproduction and feeding habits of *P. tenellus* captured in the Magela and Nourlangie Creek catchments. The mean length of specimens caught was 22.5mm. The juveniles were mostly found in floodplain lagoons and to a lesser extent in shallow lowland lagoons downstream of the Ranger Uranium Project Area. In the Magela catchment, adults were found in much the same habitats as the juveniles but a higher proportion was found in corridor and branch lagoons. In the Nourlangie catchment, juveniles and adults were found in lowland muddy channels and shallow muddy lagoons.

Although Bell *et al.* (1984) found *P. signifer* to be numerically important in a temperate tidal mangrove in Botany Bay, NSW, Morton *et al.*, (1988) did not find this to be the case in a subtropical saltmarsh (specimens only caught in autumn and summer) and suggest a cause could be the harsh environmental conditions of the semi-permanent saltmarsh pools.

Adaptability.

P. signifer's habitat is extremely variable, ranging from marine to freshwater (Allen and Cross, 1982). *P. signifer* was found in saltmarshes, where the salinity ranged from just under 20 to nearly 40 g/l (Morton *et al.*, 1988) which indicates that these fish tolerate a wide range of salinities. Gee (1988) determined that *P. signifer* was successfully able to maintain buoyancy in salinities from 0 to 60 g/l by altering swimbladder volume. He found that the blue-eye adjusted swimbladder volume in less than seven hours following a sudden change from 5 to 35 g/l. According to Gee (1988) the fish assumed a head up or down body angle (swim behaviour) and this, together with the plasticity of their swim bladder allows it to exploit the variable estuarine environment. Since salinities in estuaries, where *P. signifer* are generally found, vary considerably they must be able to cope with changes in osmotic pressure. However, in Bell *et al*'s (1984) study they were not found in spring when the salinity reached a high of 60 g/l and a low of approximately 5 g/l. They can be found in a temperature range of 15-28°C at Ph levels ranging from 5.5-7.8 (Allen and Cross, 1982). *P. gertrudae* have been found at a temperature range of 23-28°C and a Ph from 5.2-7.6 (Allen and Cross, 1982). *P. gertrudae* were found to be tolerant of acidity when maintained at pH 3.5-7.8 during laboratory breeding (Semple, 1985a). *P. mellis*, *P. gertrudae* and *P. tenellus* are all found in freshwater environments and no studies have been performed on their tolerance of saline conditions.

P. signifer has also been found in polluted water (McDowall, 1980). However, no laboratory tests have been performed on tolerances of *Pseudomugil* species to pollutants. *P. mellis* are found in nature at a temperature range of 27.5-32.2°C in soft acidic water (pH 4.4-5.8) (Allen and Ivantsoff, 1982). *P. tenellus* has been found at a temperature range of 28-35°C by Allen and Cross (1982) and 23-30°C by Merrick and Schmida (1984) and at a pH of 5.2-6.7 (Merrick and Schmida, 1984).

Diet.

Pseudomugil species appear to be opportunistic omnivores. The major foods found to be eaten by *P. tenellus* in Bishop *et al*'s (1995) Magela Creek study were algae (39%), microcrustaceans (32%) and aquatic insects (9%).

As early as 1915 it was found that *P. signifer* fed on natural food such as rotifers and had a liking for mosquito larvae (Gale, 1915). *P. signifer* surface feeds on insects (Booth *et al.* 1985) and has been reported as a successful mosquito destroyer as it swims in the shallows eliminating mosquito larvae (McDowall, 1980). A study on prey detection by *P. signifer* was carried out by Booth in 1980. He found that various insects were included in their diet in the proportions presented provided they were below a maximum size fixed by the mouth-gape size of the fish. Encounter rates were affected by prey body size and water turbidity but not by the hunger of the fish. Morton *et al.* (1988) made a field study of the seasonal abundance and feeding of fishes in a subtropical saltmarsh. *P. signifer* occurred in 9 out of the 20 samples taken throughout 1984. Their stomach fullness was found to be higher during the day in summer and during the night in autumn. They fed mainly on mosquito larvae (89% day, 33% at night) but also on larval dermapterans, larval coleopterans and arachnids during the summer. They were found to eat algae at night in summer. They ate copepods, arachnids, adult dipterans and mosquito larvae to a lesser degree than in summer (10%), although this may reflect seasonal availability of the organisms concerned. The availability of mosquito larvae would have probably been lower during autumn indicating that it was not by preference that *P. signifer's* intake was lower.

Territoriality and aggression.

P. signifer, *P. mellis*, *P. gertrudae*, and *P. tenellus* males all have been found to exhibit territorial behaviour (Semple, 1985b, 1986 and 1991). *P. mellis* males were found to be aggressive towards each other and exhibited territorial behaviour year round guarding vegetated sites from all other members of the same species (Semple, 1991). However, *P. signifer* and *P. tenellus* males became territorial only when in breeding condition (Semple, 1985b and 1986). Some individual *P. signifer* have been reported to be very aggressive (Leggett and Merrick, 1987). Males of *P. signifer* from northern Queensland were found to be extremely aggressive when more than one male was housed in an aquarium (Semple, 1986). Aggressive behaviour in male *P. mellis* often resulted in death through stress or infection following abrasion (Semple, 1991). Male *P. mellis* defended their territory after spawning and in so

doing protected fertilised eggs (Semple, 1991). Mature male *P. tenellus* have been seen to spar with other males (Leggett and Merrick, 1987). On the other hand males of *P. gertrudae* were not observed to attack other members of their species (Semple, 1985a). No information is available on interspecific aggression.

Cannibalism.

Newly spawned eggs from *P. signifer* have to be separated from adults to prevent cannibalism (Semple, 1986). *P. gertrudae* were also found to be cannibalistic towards their eggs (Semple, 1985a). *S. vermeilipinnis* kept under aquarium conditions also eat their own eggs even when plenty of food is made available (Unmack and Brumley, 1991).

***Pseudomugil* as prey.**

No studies which identify specifically *Pseudomugil* as prey have been reported. It is plausible that larger Australian native fish within the range of the species use them as prey, as with other predatory organisms such as birds.

1.2.3 Exotics.

Definition - The Concise Oxford Dictionary defines exotic as "introduced from abroad". Hence an exotic species is defined as one that is introduced into an area which is not part of its natural range.

Exotic species and how they are established.

Since the settlement of Europeans in Australia many foreign animals have been deliberately introduced and established in the wild. For a sexually reproducing species to establish itself it must overcome certain obstacles:- It must reach the new place. The new habitat must provide food, water, protection and appropriate weather conditions. A group of animals must form a minimum number of individuals to enable reproduction to occur. The genetic plasticity of the species has limits of tolerance for water quality requirements such as pH, salinity, nutrient supply and balance and turbidity (Reddacliff, 1985). It has been suggested that a successful invading species will be small in size, highly mobile with a high fecundity and a short longevity (Kitching, 1986). They must also meet some favourable interactions with other species such as species of plant or animal on which they can prey and an

absence of predators which can exploit them as a food source.

It does not follow automatically that an exotic species is a pest. For example the monarch butterfly *Danaus plexippus* (L.) is considered a non-pest exotic insect in Australia (Zalucki, 1986). Carefully introduced exotics have had favourable effect, such as *Cactoblastis cactorum* which successfully reduced numbers of prickly pear *Opuntia* spp. in Australia (Holloway, 1964). No evidence of directly positive interactions have been found for the exotic fish, *Gambusia holbrooki*. However *G. holbrooki* has been useful in the control of malarial mosquitos in some places (Das and Prasad, 1991). Although *G. holbrooki* was introduced for the purpose of lowering numbers of mosquitos in Australia there is no objective evidence that they are better than *Pseudomugil signifer* or other species of native fish species (Lloyd *et al.* 1986). Evidence from this thesis suggests that they are a threat to native fish species.

Introduced or exotic animals and plants have been known for some time to be likely to be detrimental to native ecosystems (Frith, 1973). According to Elton (1958) local extinction of native species is a common result of introduction of exotic organisms. Human-induced range extensions are a major threat to the integrity of natural communities.

These introductions have been both accidental and deliberate (Ovington, 1978). For example, plants have entered Australia on clothing, animals, grain, fodder packing materials and on vehicles. Terrestrial exotics have also been introduced deliberately, e.g., rabbits, sheep, horses, foxes and camels.

1.2.3.1 Exotic fish species.

Transfers of fish date back to Roman times (Theinemann, 1950) when *Cyprinus carpio* were brought to Greece and Italy for pond culture. From the mid 1800s to 1940 the transfer of fish increased.

Transfers of fish occurred for the following reasons (Welcomme, 1984):-

a. Sport or recreation
b. Aquaculture
c. Ecological manipulation
d. Control of unwanted organisms
e. Ornament
f. Accidental introductions

More than 160 fish species have been transferred to 120 countries (Welcomme 1984). Townsend and Winterbourn (1991) point out that the majority of introductions have gone unstudied and their effect on native fauna and flora are generally not reported or obscure. In the majority of cases it is not known whether a newly introduced exotic has integrated into the community with little effect or whether native species have been reduced in abundance or eliminated. One of the most spectacular examples of the effect of an exotic on a native fish is that of the Nile Perch, *Lates nilotica*, on Lake Victoria (Barel *et al.*, 1985; Tudge, 1990). It is not known exactly how the Nile Perch entered Lake Victoria. In 1960 it found its way into the lake possibly from ponds into which it had already been introduced and it rapidly depleted the majority of native species of cichlid fish. In New Zealand 20 exotic freshwater fish species have been introduced (Townsend and Winterbourn, 1991). New Zealand is known to have only 27 native freshwater fish and the addition of this number of exotics could have severe implications.

Cichla oecellaris, introduced into the Chagres River, Panama, proceeded to Gatun Lake and eliminated local species including the atherinid *Melaniris chagrensis*, four species of characins and two species of poeciliids (Zaret and Paine, 1973). The food web was greatly altered and simplified as the native fish species were eliminated (Welcomme, 1984). In many cases it is difficult to perceive whether the decreases in numbers are caused by competition for common resources, predation or by an interaction of both (Welcomme, 1984). The salmonids, in particular the rainbow trout and brown trout have possibly the most notorious record in causing the decline of endemic species. They have been instrumental in causing the decline of the endemic galaxiids and New Zealand grayling, *Protroctes oxyrhynchus,* from New Zealand.

Deacon *et al.* (1964) implicates direct competition for food in the reduction in numbers of the cyprinodont, *Crenichthys baileyi*, after the introduction of the guppy, *Poecilia reticulata* into the southwestern United States.

1.2.3.2 Exotic fish in Australia.

Information on the impact of introduced species and their interactions with Australian fish is generally anecdotal and piecemeal. Knowledge of the biology of native freshwater fish is incomplete. In addition, the effects of exotic species may be masked by physical modification of native fish habitats (Cadwallader, 1978). It

should be assumed that any introduction of a non native fish will have an impact on native fish species (Cadwallader, 1978).

In Australia at least four species from the family Cyprinidae (Goldfish *Carassius auratus*, European or common carp, *Cyprinus carpio*, Roach, *Rutilus rutilus* and Tench, *Tinca tinca*) were introduced, all over a hundred years ago. As well, in 1960 a new strain of the European carp, *Cyprinus carpio*, was illegally introduced to Victoria, resulting in establishment of a large population in the Murray-Darling division (Morison and Hume, 1990). Although no conclusive evidence was found of an association between carp densities and turbidity, there was strong circumstantial evidence that carp had reduced the density of shallow-rooted and soft leaved aquatic vegetation (Fletcher *et al.* 1985). It has also been suggested that *C. carpio* modify the habitat in the course of their feeding and spawning behaviour (Wager and Jackson, 1993), which would have implications for native fish.

Five species of the family Salmonidae have been introduced to Australia (Quinnat or Chinook Salmon, *Onchorhynchus tshawytscha*; rainbow trout, *Onchorhynchus mykiss*; Atlantic salmon, *Salmo salar*; brown trout, *Salmo trutta* and brook char, *Salvelinus fontinalis*). They are thought to have restricted ranges governed by thermal tolerance (McDowall and Tilzey, 1980; Merrick and Schmida, 1984).

Both the brown trout (*S. trutta*), introduced to Australia in 1864, and the rainbow trout (*O. mykiss*), introduced in 1894, have been able to establish themselves by finding favourable habitat and successfully reproducing. Both have been implicated in the decline of *Galaxias fontanus* and *Galaxias olidus* in drainages where they have been introduced below natural barriers (Tilzey, 1976; Jackson and Williams, 1980). They are thought to eliminate small native fish because they are voracious predators. *Salmo trutta* were thought to detrimentally affect the native New Zealand galaxiid, *Galaxias vulgaris* (McIntosh *et al.* 1992). *Salmo trutta* forced *G. vulgaris* into less favourable feeding locations suggesting that there was interspecific competition for space. This, combined with predation by the trout and competition for food could be the cause of reduced populations of *G. vulgaris*. Money is gained from the tourism industry, as trout are sought-after sport and table fish. However, they are damaging exotics in that they compete for food and actively hunt small native fish and crayfish (Tilzey, 1976, Romanowski, 1992).

One species from the family Percidae, namely *Perca fluviatilis*, was released in south eastern Australia over a hundred years ago and has a patchy distribution

restricted only by temperatures above 31°C and high stream velocities (Merrick and Schmida 1984). These authors also describe four species of cichlids (Convict or Zebra Cichlid, *Cichlasoma nigrofasciatum*; Jack Dempsey Cichlid, *C. octofasciatum*; Black Mangrove or Niger Cichlid, *Tilapia mariae* and Mozambique Cichlid or Mouth-brooder, *Oreochromis mossambicus*) which have been established in Australian waters.

Even though cichlids have been introduced to Queensland, Victoria and Western Australia very little biological data are available on their interaction with native fish (Wager and Jackson, 1993). *Oreochromis mossambicus* has not been shown to have dietary overlap with the Spangled Perch, *Leiopotherapon unicolor* or the Eel-tailed Catfish *Tandanus tandanus* (Blüdhorn *et al.*, 1990). Their research did not look at whether interactions, such as predation, occur between *O. mossambicus* and Australian native fish.

The oriental weather loach (*Misgurnus anguillicaudatus*) was thought to have been introduced from escaped aquarium fish. In 1989 they were found to be widespread and reproducing well in Wingecarribee River, NSW (Burchmore *et al.*, 1990). It has been speculated that they interact with natives through competition for spawning sites, disturbance or predation of eggs and competition for food or shelter and alteration of habitat (Lintermans *et al.*, 1990).

Red fin, *Perca fluviatilis*, were first introduced to Australia in 1862 and compete for food and space with native Murray cod (*Maccullochella peeli*) and Golden Perch (*Macquaria ambigua*). In addition they have been implicated in the reduction of Macquarie perch (*Macquaria australasica*) in Lake Eildon in Victoria (Cadwallader and Backhouse, 1983).

1.2.3.3 *Gambusia holbrooki* in Australia.

A number of poeciliids have become established in Australian waters (Guppy *Poecliia reticulata*, swordtails *Xipophorus* spp., *Gambusia holbrooki* and *Gambusia dominicensis*) (Merrick and Schmida, 1984). These are small fish but this does not mean that they cause less damage than large species. *Gambusia holbrooki* only grows to a maximum of 60mm in length (Merrick and Schmida, 1984) but has been cited several times as being of pest status within Australian waterways (Lloyd, 1982; Arthington *et al.*, 1983 and Merrick and Schmida, 1984).

G. holbrooki is able to reproduce rapidly when environmental conditions are

favourable. In contrast, *P. signifer* does not have such a high reproductive potential (Morton *et al.* 1988). It is for this reason that *G. holbrooki* is considered to have adverse effects on native species (Myers, 1965; Arthington *et al.*, 1986).

Reasons for introduction to Australia.

G. holbrooki was introduced to Australia for mosquito control. The two closely related species *G. holbrooki* and *G. affinis* have been introduced to many other parts of the world for the same reason. In a review of introduced poeciliids Courtenay and Meffe (1989) found that the majority of evidence suggests that *G. holbrooki* and *G. affinis* are unsuccessful in mosquito control (Grant, 1978; Lake, 1971; Moyle, 1976; Danielson, 1968; Walters and Legner, 1980; Sharma and Al-Daham, 1979; Hildebrand, 1930; and Pflieger, 1975). Modest control of mosquitoes has been reported by Cech and Linden (1986, 1987). Mosquito control by *G. affinis* has been observed (Das and Prasad, 1991; Nelson and Keenan, 1992). Das and Prasad (1991) reported that at a stocking density of 5 *G. affinis* per square metre significantly reduced the larval and pupal densities of mosquitos in rice fields in India. Castleberry and Cech (1990) found guppies (*P. reticulata*) superior to *G. affinis* and Pupfish (*Cyprinodon nevadensis amargosae*) for mosquito control mainly due to the rapid increase in the abundance of *P. reticulata*. More recently Lardeux (1992) conducted a study which used *G. affinis* and *P. reticulata* in conjunction with the copepod *Mesocyclops aspericornis* for mosquito control in a French Polynesian village. The fish appeared to successfully eliminate the mosquito larvae from the open breeding ponds. They concluded, however, that the trial had been unsuccessful as the biting rate of *Aedes aegypti* was not reduced. Full eradication may not have occurred because the copepod was not able to eliminate the mosquito larvae from drums and other water containers.

Nelson and Keenan (1992) conducted a study in Colorado, USA comparing the effect of mosquito control by *G. affinis* (introduced) to the native *Fundulus zebrinus*. They concluded that the native fish and *G. affinis* reduced numbers of mosquito larvae to the same level. They support the usage of native species over the introduced species for both economic and environmental reasons. They suggest that the native fish populations that had previously acted as larvivores had been reduced in numbers by the introduction of the exotic species. Australia is now in a similar position. Anecdotal evidence suggests that *G. holbrooki*, introduced for mosquito

control, may be detrimental to the native fish that could have acted as natural larvivores. Even though Morton *et al.*, (1988) found *G. holbrooki* to be more successful at mosquito control than native fish in a subtropical Australian saltmarsh, they did not recommend them as a useful tool for mosquito culling, as the number of mosquitofish that would be required to eradicate mosquitoes would be prohibitive and they would have a detrimental impact on native species. They think it improbable that any fish species could singly eradicate the mosquito larvae unless the fish were supplied annually at the same time as mosquito hatching. *G. holbrooki* have been considered no more efficient than other native Australian insectivorous fish (e.g. *Craterocephalus, Retropinna, Melanotaenia, Hypseleotris, Mogurnda* and *Galaxias*) for mosquito control (Grant, 1978; Lake, 1971; McDowall, 1980; Lloyd, 1984 and Merrick and Schmida, 1984). However, no data have been published on their effectiveness. Lloyd (1990a) claims that interactions between *G. holbrooki* and native fish are intense and include competition, predation and hybridisation. Hybridisation between introduced poecilids and Australian native freshwater fish is not possible. *G. holbrooki* is regarded as a competitive threat to Australian native fish (Lloyd, 1982; Arthington *et al.*, 1983). Arthington *et al.*, (1983) consider that there is competition for food and space between *G. holbrooki* and *P. signifer*. If this is the case it would have definite implications for the status of *P. signifer*. *G. holbrooki* is known to eat fish eggs and juveniles, and aggressively fin nip other fish (Koehn and O'Connor, 1990). They are thought to be responsible for the extinction of several fish species in Africa and South East Asia (Cadwallader and Backhouse, 1983). No extinctions due to *G. holbrooki* in Australia have been documented (Koehn and O'Connor, 1990).

1.2.4 Members of the genus *Gambusia*:- *Gambusia holbrooki* and *Gambusia affinis*.

1.2.4.1 Taxonomy.

Gambusia holbrooki belong to the family Poeciliidae, order Cyprinodontiformes (Rosen and Bailey, 1963). There are approximately 30 species within the genus *Gambusia* (Rivas, 1963). There has been some confusion as to the taxonomic position of the eastern Australian *Gambusia*. Most authors prior to 1985 named the fish found in Australia as *G. affinis*. They originally came from the south-eastern USA, west to Texas (Lloyd and Tomasov, 1985). Two subspecies of *G. affinis* were

identified in the south-eastern USA: the eastern form *G. affinis holbrooki* (Krumholtz 1948) and the western *G. affinis affinis* (Baird and Girard 1853).

Lloyd and Tomasov (1985) identified the Australian found *Gambusia* sp. as the subspecies *G. a. holbrooki* derived from Georgia (U.S.A.) via Italian stocks in 1926 (Wilson, 1960). Wooten *et al.* (1988) later separated the subspecies *G. affinis holbrooki* and *G. affinis affinis* into two species namely *G. holbrooki* (Girard, 1859) and *G. affinis* (Baird and Girard, 1853) as they were originally described. The species found in Australia is presently named *G. holbrooki*. Both *G. affinis* and *G. holbrooki* have been commonly named the mosquito fish but Lloyd (1990b) suggests that they should be referred to under their scientific name. He suggests that *G. holbrooki* be commonly called the eastern gambusia to detract from the suggestion that they are any better than any other fish at controlling mosquitos. It is likely that many studies using *G. holbrooki* were reported to have used *G. affinis*, due to the persistent taxonomic confusion between the two similar species. In referring to non-Australian studies the species cited (*G. holbrooki* or *G. affinis*) are accepted as named. However all references to either *G. holbrooki* or *G. affinis* studies in Australia will be referred to *G. holbrooki*. This convention is followed throughout this thesis.

1.2.4.2 Distribution.

As a consequence of a mosquito-control program in the 1920s, the genus *Gambusia* had the largest distribution of any freshwater fish in the world in the late 1940s (Krumholtz, 1948).

In Australia *G. holbrooki* is found in at least eight drainage divisions (Fig. 1.2) (Merrick and Schmida, 1984; Arthington and Lloyd, 1989). Changed environments, often created by humans, appear to be susceptible to invasion by these fish (Courtenay and Meffe, 1989; Moyle, 1976, Arthington *et al.*, 1983). In a review, ten cases were cited world wide in which *G. holbrooki* or *G. affinis* were successful in modified habitats (Courtenay and Meffe, 1989).

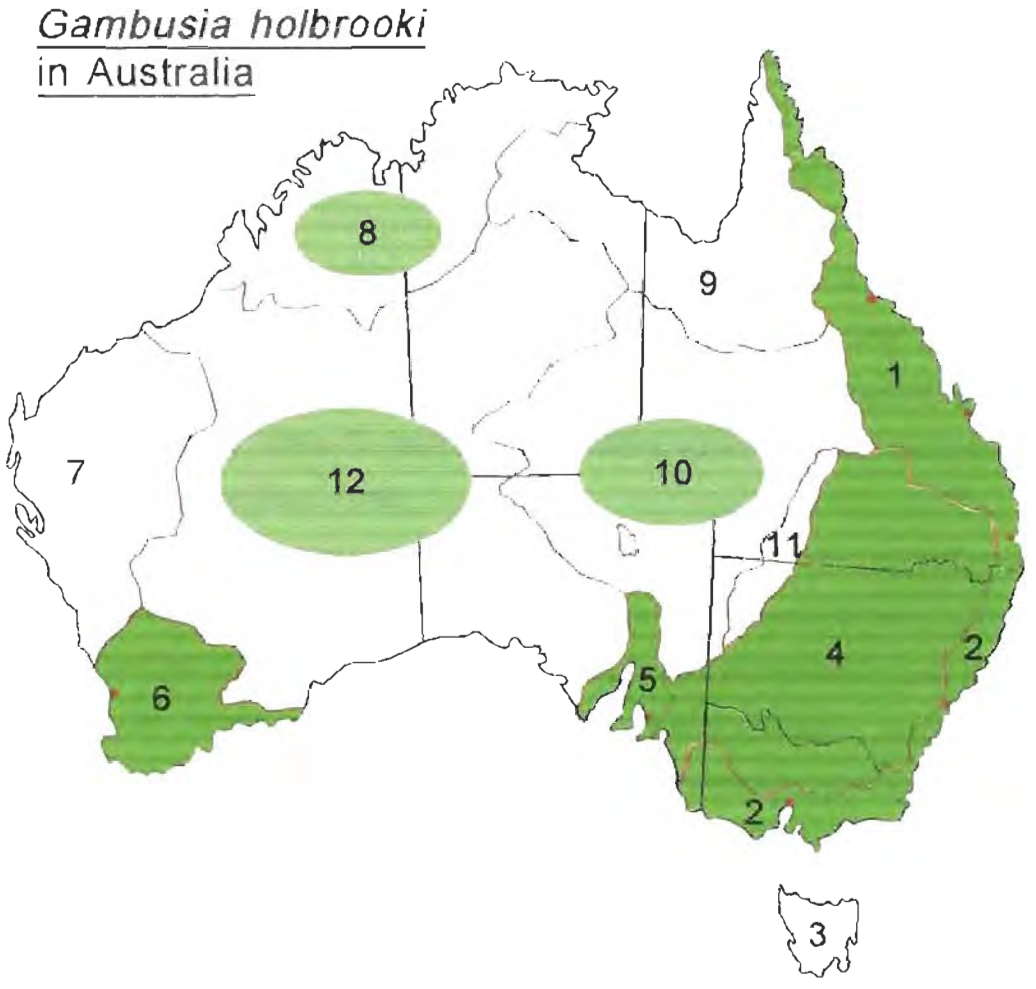


Figure 1.2 Distribution of *Gambusia holbrooki* in Australia.
(After Merrick and Schmida, 1984; Arthington and Lloyd, 1989)
Key to divisions where *G. holbrooki* are found.

Widely distributed.	Restricted distribution.
1. North East Coast.	8. Timor Sea.
2. South East Coast.	10. Lake Eyre Division.
4. Murray/Darling.	12. Western Plateau.
5. South Australian Gulf.	
6. South West Coast.	

1.2.4.3 Description.

The family Poecilidae can be identified by an upturned mouth, large scales, no lateral line, one soft-rayed dorsal fin and a gonopodium in males (Rosen and Bailey, 1963 and Merrick and Schmida, 1984). *Gambusia* species are identified by gonopodium structure and meristic characters (Lloyd and Tomasov, 1985).

Reproduction.

The growth and reproduction of *G. holbrooki* and *G. affinis* is much better documented than small native Australian fish. Female *G. holbrooki* can reach a length of 60 mm but males only grow to 35 mm (McDowall, 1980). The anal fin and pelvic extension of male *G. holbrooki* is modified to form an intromittent organ, the gonopodium (Constantz, 1989).

Paired testes are fused into a single tubular organ attached to the dorsal body wall (Rosen and Bailey, 1963). Tubules surround a central lumen (Billard, 1986) and spermatogonia pass down the tubules towards the centre of the testis and join with Sertoli cells to form cysts (Harrington, 1974) which become sperm bundles (Philippi, 1908). Spermatogenesis is dependent on environmental factors such as daylength and temperature (Constantz, 1989).

Females have paired ovaries that are unified into a single organ which is dorsally suspended in the body cavity (Rosen and Bailey, 1963 and Wourms, 1981). During vitellogenesis the oocyte enlarges (Amoroso, 1960) and is surrounded by epithelial cells to form an ovarian follicle (Hoar, 1969) from which the embryo develops. Female *G. holbrooki* have a reproductive opening on the end of a large papilla within an open urogenital sinus (Peden, 1972). A black spot composed of a concentration of melanophores develops near the anus in the female (Medlen, 1951) at sexual maturity and is largest when eggs are present in the ovaries, and inconspicuous when the young are ready for birth (Peden, 1973). It has been suggested that it provides a cue for gonopodial orientation during fertilisation by the male (Peden, 1973). *G. holbrooki* has denticles on the back surface of ray 3 and an unsegmented bony claw on ray 4p of the gonopodium whereas *G. affinis* does not have denticles on ray 3 and has a segmented claw on ray 4p (Lloyd and Tomasov, 1985).

G. holbrooki are considered lecithotrophic livebearers (Constantz, 1989). Lecithotrophy includes the older term ovoviviparity (Amoroso, 1960) as the eggs are retained and nutrition of the embryo is entirely derived from the yolk (Petrovický, 1988).

Fertilisation is internal and females can hold live sperm in the ovaries for several months or up to eight broods in some poeciliids (Turner and Snelson, 1984). Females are thought to nourish the sperm within the lining of the ovary (Hoar, 1969). When the ovarian epithelium touches the follicular wall, sperm infiltrate cells of the weakened follicle (Amoroso, 1960). The females usually have both developing embryos and maturing unfertilised eggs in their ovary (Vondracek *et al.*, 1988). The eggs are fertilised as soon as the preceding young are hatched. The gestation period is from 3-4 weeks and the young are a few millimetres in length at birth (Krumholtz, 1948; Merrick and Schmida, 1984). They are able to reach sexual maturity in 2 months when both sexes are 22-23mm standard length (Merrick and Schmida, 1984).

The species produces several broods of approximately 50 young several times during the summer months (McDowall 1980, Trendall, 1982). Their reproductive potential is therefore prodigious. Their peak breeding time has been found to be at the beginning of the breeding season (Milton and Arthington, 1983). Female poeciliids are known to release a pheromonal compound, possibly an estrogen, that promotes male sexual behaviour (Amouriq, 1964, 1967; Constantz, 1989). The pheromone is perceived by the male by taste after the females have produced a brood (Parzefall, 1973).

1.2.4.4 Ecology.

G. holbrooki occupies a wide range of habitats from temperate to tropical areas (including many different sized water bodies from fresh to saline conditions with constant to chemically harsh environments) (Meffe and Snelson, 1989b). They are generally found in small shallow bodies of water or on the margins of larger bodies, in slow moving water with partial or heavy aquatic vegetation (Meffe and Snelson, 1989b). When provided with a choice, *G. affinis* were found to move to submerged vegetation rather than open water (Casterlin and Reynolds, 1977). *G. holbrooki* is

thought to select shallow waters for the following reasons:- to reduce the possibility of predation (Goodyear, 1973), substrate selection for parturition (Endler, 1980, 1982) oxygen availability (adaptability to low dissolved oxygen (Cech *et al.*, 1985)) and temperature (Maglio and Rosen, 1969). *G. affinis* have been found to use the sun to orient towards shallow marginal habitats away from predators in the deeper water (Goodyear, 1973).

Adaptability.

G. holbrooki is extremely adaptable (Meffe and Snelson, 1989b). They can survive temperatures from 0.5°C to 38°C (Otto, 1973). They have also been found in lakes at temperatures of 42-44°C (Ferrens and Murphy, 1974). However, genetic differences in capacity for adaptation to critical thermal maxima and lower lethal temperatures have been demonstrated for *Gambusia affinis* (Otto, 1973). In addition, smaller females have been found to be more tolerant to high temperatures than males (Winkler, 1975, 1976). *G. affinis* are found in freshwater and estuarine environments (Stearns and Sage, 1980) and *G. holbrooki* have been found in saltwater with salinities in excess of 30 g/1 (Chessman and Williams, 1974). However, from life history and growth rate studies it has been suggested that *G. affinis* is best adapted to freshwater (Stearns and Sage, 1980 and Zimmerer, 1983).

G. affinis can tolerate extremely low levels of oxygen. The 96 h LC50 value for oxygen is approximately 0.2 mg/l (Sjogren, 1972). They also tolerate many pollutants including insecticides, (Culley and Ferguson, 1969) herbicides, (Johnson, 1978) rotenone, (Fabacher and Chambers, 1972) phenol, (Meynell, 1973) radiation (Blaylock and Mitchell, 1969, Blaylock, 1969) and heavy metals (Kania and O'Hara, 1974). Organochlorine insecticides were less toxic to *G. affinis* than synthetic pyrethroids, and organophosphorus insecticides were less toxic than organochlorine insecticides (Mittal *et al.*, 1991). A microbial insecticide ABG-6262 was not toxic after a weeks exposure at 2.5 mg/l (Mittal *et al.*, 1991). However, these authors did report that many chemical insecticides were able to abort the young of gravid mosquito fish.

In poeciliids there appears to be more females than males in wild populations (Snelson, 1989). The uneven sex ratio in adult wild *G. affinis* has been attributed to

the more significant impact of stressors, such as temperature extremes, overcrowding and starvation, on males (Krumholtz, 1948).

Diet.

G. holbrooki is primarily a carnivore (Merrick and Schmida, 1984 and Booth, 1980) although it has been considered an omnivore with a preference for animal food (Farley, 1980). They have strong conical teeth, cephalic sensory canals and short guts, typical of predators (Meffe *et al.*, 1983; Turner and Snelson, 1984 and Rosen and Mendelson, 1960). In their study of fish in an Australian subtropical saltmarsh Morton *et al.* (1988) found that mosquito larvae were the most important food (42% day and 53% night) in summer. They also ate dipteran adults, coleopteran larvae, adult hymenopterans, dermapterans and hemipterans. Mosquito larval intake was reduced to 5.8% in the day and 15% at night in autumn. Unlike *P. signifer*, the diet of *G. holbrooki* when caught in winter was very different to that in summer. Crustaceans made up the major component (87.5%) of the diet. Fish eggs or larvae were not reported in their stomach contents by Morton *et al.* (1988) but they are known to eat fish eggs and juveniles (Koehn and O'Connor, 1990).

G. affinis grew better in terms of weight when fed tubifex worms rather than mosquito larvae in a laboratory experiment (Reddy and Shakuntala, 1979). In addition when juvenile *G. affinis* were provided with either brineshrimp nauplii or tubifex worms to satiation it was found that those fed the brineshrimp nauplii were 22% heavier at 25 days of age (Wurtsbaugh and Cech, 1983).

Territoriality and aggression.

No documented evidence of territoriality was found for *G. holbrooki*, and poeciliids generally have not been observed to display territorial behaviour, except for a doubtful case of the "home cage effect" reported in female swordtails (*Xiphophorus*) by Heuts (1968). Lloyd (pers. comm.) subscribes to the view that *G. holbrooki* are not territorial. Although *G. holbrooki* does not defend specific territory, the species is known to be very aggressive, attacking and nipping the fins of other fish (Minckley and Deacon, 1968; Grant, 1978; McDowall, 1980; Lloyd, 1984; Lloyd, 1990b and Wager and Jackson, 1993).

Predation and cannibalism.

G. holbrooki and *G. affinis* have a great impact on community and ecosystem structure through their predatory habit (Courtenay and Meffe, 1989). Predation by *G. affinis* on the native Sonoran topminnows (*Poeciliopsis occidentalis*) in south-western USA eliminated the topminnow in many areas (Meffe *et al.*, 1983; Meffe, 1985 and Courtenay and Meffe, 1989). It has been suggested that predation on juveniles of other species is intense and can lead to replacement by *G. affinis* (Myers, 1965). *G. affinis* have eliminated *Cyprinodon calaritanus* (Missiroli, 1948) and *Rhinichthys osculus* in southern Nevada (Deacon *et al.*, 1964).

Contrary to a previous report on other fish species (Cook and Streams, 1984), Linden and Cech (1990) in their study on *G. affinis* observed that the presence of plant material increased the number of prey that they were able to eat.

Cannibalism has been suggested to aid in survival during food shortages, to remove possible competitors or predators, and can be a means by which a population is regulated (Fox, 1975 and Polis, 1981). It was found through laboratory experimentation (providing dried food supplemented with a crustacean or unrelated teleost or adult or juvenile conspecifics) that cannibalism by *G. affinis* can help provide nutrients leading to better growth and reproduction in comparison with those individuals that do not cannibalise conspecifics (Meffe and Crump, 1987). *G. affinis* and *G. holbrooki* have been found to be cannibalistic in the wild (Krumholtz, 1948; Harrington and Harrington, 1982 and Nesbit, 1993). However, Nesbit (1993) found cannibalism of neonate or juveniles to be very low in the wild (1.15%) and warns against the use of laboratory results for drawing conclusions of an evolutionary nature and on selective benefits.

***G. holbrooki* or *G. affinis* as prey.**

In North America, predatory fish, wading birds, snakes and invertebrates have been found to prey on *G. affinis* (Hunt, 1953; Mushinsky and Hebrard, 1977 and Meffe and Snelson, 1989b). It is thought that poeciliids may increase their reproductive effort in response to heavy predation (Meffe and Snelson, 1989a). In Australia possible fish predators of *G. holbrooki* include species of *Anguilla*, *Mogurnda*, *Gobiomorphus*, *Leiopotherapon* and *Glossamia*, but no systematic studies

have been performed on their effect on *G. holbrooki* in the wild (Lloyd *et al.*, 1986). Lloyd (1990a) has asserted that both native and introduced fish predators prefer to avoid feeding on *Gambusia spp.*

1.2.4.5 Reasons for the success of *G. holbrooki* and *G. affinis* following introduction.

G. holbrooki and *G. affinis* have been extremely successful as introduced species (Courtenay and Meffe, 1989). Courtenay and Meffe (1989) suggest that they fit seven of the eight criteria selected by Ehrlich (1986) for successful invasion, namely: abundant in original range; are polyphagous; have a short generation time; a single inseminated female can colonise a new site; broad physiological tolerances, are closely associated with humans; and have high genetic variability. In addition, two more criteria for success have been proposed by Courtenay and Meffe (1989). First they have specialised reproduction, i.e. producing reasonable numbers of well developed young numerous times per year having merely broad temperature and daylength requirements to reproduce and the newly hatched fish being independent of the adults after birth. Secondly, adults, in particular the females, are extremely aggressive and fin nip, often causing the death of other species (Meffe, 1985). Predation pressure on young of other species is extreme (Myers, 1965).

1.3 Competition.

A number of ecologists have proposed definitions of competition but they all present difficulties since they usually contain terms whose meanings are themselves obscure or open to debate (Arthur, 1987). Arthur restricts the term to situations where species are mutually inhibitory to each other and uses the symbols (-,-) for a competitive interaction between two species. Other types of interactions he says must be represented by other combinations of symbols where (+) represents a stimulatory effect and (0) no effect.

Keddy (1989) points out that definitions of competition present a challenge; that there is no universal agreement on the matter, and that some authors no longer even use the term. However for the purposes of his ensuing critique of the subject he defines competition as "the negative effects which one organism has upon another by

consuming or controlling access to, a resource that is limited in availability" (Keddy, 1989). A number of investigators have carried out experiments to determine whether competition occurs between different fish species (Fausch and White, 1981; Jones, 1987 and 1988; Persson and Greenberg, 1990; Glova and Sagar, 1991; McIntosh, *et al.*, 1992 and Douglas *et al.*, 1994). Competition has been divided by Lloyd (1990a) into resource competition (for space and food) and interference competition, (for example fighting) when one individual directly affects another (Keddy, 1989). Interference by individuals of the same species (intraspecific) may be evenly matched and thus have no significant effect on the population. Interspecific interference may occur between species which are ill-matched causing the dominant species to displace the other species (Pontin, 1982).

Lloyd (1990a) suggests that there is evidence that *G. holbrooki* exhibits interference competition as they have effects on the distribution, relative abundance and growth or survival on surrounding species. Pontin (1982) says that interspecific competition is "an interaction between two (or more) species which results in reduced population size of both (or all) competing species". The present study has been particularly concerned with elucidating whether *G. holbrooki* has a detrimental affect on the growth and reproductive success of *P. signifer*. If the populations of both species are not reduced in size they would not meet the criteria of either Arthur's (1987) or Pontin's (1982) concept of competition.

1.4 Objectives of present study.

The overall goal of the current study was to provide information on the basic reproductive biology of *P. signifer*, and the impact of water quality parameters and the presence of the exotic species *G. holbrooki* on the growth and reproductive capacity of *P. signifer*. Survival strategies used by *P. signifer* and *G. holbrooki* are compared.

1.4.1 Implications for management.

Freshwater fish are important from an aesthetic point of view. They are also of great use as biological monitors (Williams, 1980). However, very little research has been carried out on the management of Australian freshwater fish although Australia

needs a management plan to protect its small native fish species. Before management policies can be developed, it is necessary to have a basic understanding of the biology and ecology of the fish (Koehn and O'Connor, 1990), and the impacts of change in water quality upon them and the presence of exotic biota.

It has been suggested that *G. holbrooki* invades disturbed habitats (Arthington *et al.*, 1983) and they have been implicated in detrimental effects on native fish (Myers, 1965; Schoenherr, 1981; Arthington and Lloyd, 1989). The impact of *G. holbrooki* in Australia is undocumented (Koehn and O'Connor, 1990). However, when *G. holbrooki* and another poeciliid, *Xiphophorus maculatus* were absent, *P. signifer* comprised 73% of the fish caught from College's Crossing on the Brisbane River in 1977. In contrast, in 1981 both exotic species were present and *P. signifer* comprised only 1.5% of the fish caught (McKay, 1984). *G. holbrooki* has very recently been implicated in the decline in the density of *Galaxiella pusilla* (dwarf galaxias) which is distributed in coastal Victoria, south-eastern South Australia and Tasmania (Unmack and Paras, 1995). However, no controlled experiments have been designed to determine the effect of *G. holbrooki* on our native fish and evidence so far available has been therefore circumstantial.

1.5 Experimental objectives.

The major objective of this project has been to identify factors affecting the reproductive biology of *P. signifer*. *P. signifer* was specifically used in this study because they are an easily acquired and maintained Australian freshwater fish species. The information gained can be used as a model in the investigation of more vulnerable Australian species of *Pseudomugil* with restricted ranges, which are also under threat. In particular, the experimental objectives of this investigation have included the following -

A. To examine the surface structure of the eggs by scanning electron microscopy of four Australian species of *Pseudomugil* to verify taxonomic relationships (Chapter 2). Before any management plan for a species can be put in place, as much biological information as possible should be acquired (Wager and Jackson, 1993; Koehn and O'Connor, 1990). As part of any such information it is necessary to understand the relationships between related species. This is

especially so if it is hoped to extend findings to related species. Scanning electron microscopy has not previously been used on the eggs of Australian freshwater fish species for taxonomic purposes, although it has been used as a taxonomic tool in other countries (Hagstrom and Lonning, 1968; Lonning, 1972; Lonning and Hagstrom, 1975; Stehr and Hawkes, 1979; Brummett and Dumont, 1981; Kosmath *et al.*, 1981; Morin and Able, 1983; Riehl and Kock, 1989; Mooi, 1990).

B. To study the breeding behaviour and normal embryology of four Australian species of *Pseudomugil* (Chapter 2). Although individual studies on breeding behaviour and embryology of Australian *Pseudomugil* species have been reported (Terciera, 1983; Merrick and Schmida, 1984; Semple, 1985a and 1985b; Semple, 1991), no comparative studies of *Pseudomugil* species under controlled conditions have been performed.

C. To determine whether *P. signifer* has a seasonal reproductive cycle and whether there is any relationship between reproductive activity and basic physico-chemical parameters and daylength (Chapter 3) and in addition, whether reproductive development occurs in the same manner in captivity as in the field. If reproductive development was found to be similar in the captive and field caught fish, routine studies in captivity could be more conveniently undertaken. Field studies were performed on the only *Pseudomugil* species found in NSW (*P. signifer*), with a comparison of two different catchments. A parallel study was performed on captive populations of *P. signifer* to assess whether the captive populations behaved and reproduced in a similar manner to those in the wild. No previous research has been published on the reproductive cycle of *P. signifer* in the wild.

Possible factors contributing to *P. signifer*'s sexual maturation (or cycle) were considered. Monthly samples were collected in 1985 on two major factors (salinity and temperature) and related to an examination of the patterns of gonadal maturity in *P. signifer*. In this study it was found that *P. signifer* were more abundant at the site where no *G. holbrooki* were caught. Although other factors could be implicated, such as differences in microhabitat, the work of other authors suggested that *G. holbrooki* might

have a particular detrimental effect on *P. signifer*, although this had not been demonstrated elsewhere.

D. Following from the investigations above, to resolve whether *P. signifer* is threatened by *G. holbrooki*. Tank experiments were devised to assess the effect that the exotic *G. holbrooki* might have on growth, fecundity and reproduction of *P. signifer* in a controlled, confined environment (Chapter 4). Implications for other *Pseudomugil* species (e.g. *P. mellis* and *Scaturiginichthys vermeilipinnis*) with a more restricted habitat range are discussed. This type of experiment has not been previously conducted with native Australian fish species.

E. To complement the controlled tank study in the previous section, a study was conducted to determine whether fecundity in *P. signifer* is affected by habitat conditions and by the presence of *G. holbrooki* in the field (Chapter 5). Four sites in the Sydney Basin were selected for this study, two of which had both species of fish and two of which had only *P. signifer*. It was anticipated that the data collected would provide further information on the effect of the physical and chemical environment and the possible impact of *G. holbrooki* on the fecundity and success (measured as abundance, and growth) of *P. signifer*.

Very little is known about our smaller native freshwater species of fish. This study was carried out to increase our knowledge of aspects of the biology and reproductive characteristics of *P. signifer*. The information gained is not only of interest from a biological perspective but will be of value for management and conservation purposes.

Information obtained from all the above studies could be extrapolated for the conservation of other Australian pseudomugilid species that are under threat. The information has implications for the development of future conservation management strategies on this continent.

Chapter 2.

Comparative study of reproductive biology of genus *Pseudomugil* (Pisces: Melanotaeniidae).

2.1 Introduction.

Chapter 1 reviewed the information available concerning the general biology of the genus *Pseudomugil*. This section deals specifically with aspects of the reproductive biology of the genus. *P. signifer* and *P. mellis* are popular aquarium species and an account of the courting display of *P. mellis* has been given by Terciera (1983). Merrick and Schmida (1984) briefly described the spawning behaviour of *P. signifer*. Semple (1985 a and b) has published brief accounts of the maintenance, reproduction and early development of the tropical *P. gertrudae* and *P. tenellus*. Semple (1991) described the reproductive behaviour and early development in *P. mellis*. The development of fry was described. Semple (1991) found evidence of variation between mainland populations of *P. mellis* and those described from Fraser Island by Howe (1987). These variations including non-breeding colouration, prespawning behaviour, preferred spawning site, egg size and morphology, developmental rate, embryo pigmentation and age, and stage of development at hatching may indicate divergence of geographically isolated populations of *P. mellis*. Isolating mechanisms already appear to operate to separate *P. mellis* and *P. signifer* which are sympatric and closely related (Semple, 1991).

Semple (1991) reported shifts in population numbers of *P. mellis* and related this to flooding which flushed large numbers of fish into intertidal areas. Specimens were collected at temperatures from 14-38°C (a larger temperature tolerance than previously reported). Egg size was found to be dependent on the size of the female fish. The number of filaments on the chorion overlapped between species. Semple (1991) concluded that reproductive factors should be included in systematic analyses only when the biological and abiotic processes controlling their variability have been extensively studied. It is questionable that every aspect of the animal should be known in order to comment on their taxonomic relationships within a group.

The current study has sought to answer two questions with respect to the reproductive biology of this genus:

1. Are the egg surface characteristics consistent throughout the range of *P. signifer* and between three other Australian species of *Pseudomugil*?

An attempt was made to elucidate discrepancies in the taxonomic status of the northern and southern populations. For comparative taxonomic purposes, and because it is necessary to understand relationships between species before any biological and ecological studies can be performed, the surface structure of *P. signifer* eggs was compared with that of the eggs of three other Australian *Pseudomugil* species. For the first time a scanning electron microscope has been used to study the surface structure of eggs from the genus *Pseudomugil*. Elsewhere the scanning electron microscope has been used successfully as a taxonomic tool distinguishing fish eggs to the species level (Hagstrom and Lonning, 1968; Lonning, 1972; Lonning and Hagstrom, 1975; Stehr and Hawkes, 1979; Riehl and Schulte 1977, 1978; Riehl 1980; Brummett and Dumont, 1981; Kosmath *et al.* 1981; Morin and Able, 1983; Riehl and Kock, 1989; Mooi, 1990). No previous studies of this type have been conducted on Australian freshwater species.

2. Is breeding behaviour and egg morphology in *P. signifer* similar to three other Australian species of *Pseudomugil*?

P. signifer are found in both tropical and non-tropical waters and here biological comparisons have been made with other, more restricted species of *Pseudomugil* found in tropical or sub-tropical waters. No directly comparable controlled embryological studies have been conducted on the different populations of *P. signifer* and other Australian *Pseudomugil* species. Understanding the mechanism of breeding and egg development of these *Pseudomugil* species will also provide fundamental information on how *Gambusia holbrooki* might be able to affect *Pseudomugil* species. Determining the impact of *G. holbrooki* on *P. signifer* is part of the aim of this thesis.

A preliminary examination of the chorion of the eggs was made with the scanning electron microscope (SEM). On the basis of these observations inferences were drawn about the taxonomic position of the genus. Fine structures observed can aid in discerning relationships between different species.

A comparison of spawning behaviour and colouration of *P. signifer* compared with *P. mellis, P. tenellus*, and *P. gertrudae* was also conducted. The developing eggs

and larvae were observed and comparisons made between *P. signifer* and the other three species. Larval development of *P. signifer* from four different populations (Sydney, NSW, Lake Hiawatha, NSW. Townsville, Queensland and Cairns, Queensland) was examined. Apart from assisting in a review of this genus, information obtained on these larvae has the potential of practical use for identifying larval *Pseudomugil* in field collections.

2.2 Materials and Methods.

These studies were commenced at Macquarie University, Sydney, NSW and completed at the University of Technology, Sydney, NSW.

2.2.1 Collection sites.

Adult specimens were collected from locations as described in Table 2.1.

Table 2.1. Collection sites for species of *Pseudomugil.*

Species	Site	
P. signifer	Deep Creek, Narrabeen, N.S.W.	33° 42'S 151° 15'E.
P. signifer	Smiths Lake, N.S.W.	32°23'S, 152°28'E.
P. signifer	Ross River Townsville, Queensland.	19°16'S, 146°49'E.
P. signifer	Tuggerah Lakes N.S.W.	33°20'S 151°28'E.
P. signifer	Lake Hiawatha N.S.W.	29°49'S 153°15'E.
P. signifer	Harvey Creek Cairns, Queensland.	17°16'S 145°56'E.
P. mellis	Fraser Island, Queensland.	25°22'S, 153°07'E.
P. tenellus	Ja Ja Billabong, Northern Territory.	12°40'S, 135°15'E.
P. gertrudae	Radon Hill, Northern Territory.	12°45'S, 132°54'E.

The fish from these collecting sites were used as breeding stock. Fig. 2.1 shows a map of the sites used for collection. They were collected within their distribution range (See Fig. 1.1).

Figure 2.1 Location of the sites where *Pseudomugil signifer*, *Pseudomugil mellis*, *Pseudomugil gertrudae* and *Pseudomugil tenellus* were sampled.

2.2.2 Collection of fish.

Fish specimens were collected with a two poled hand net (4mm mesh size) or with a 10mm mesh, 7.5m seine net. Specimens caught in the Northern Territory were initially placed in large plastic bags filled with water from the capture site and transported to a laboratory at Jabiru East where they were transferred into small aquaria (up to 10 fish in each). Each aquarium was topped up with water and each day one third of the water was exchanged until the fish were transported to Sydney. Specimens that were collected other than in the Northern Territory were transported to Sydney in large plastic bags with aeration provided by a battery operated pump.

2.2.3 Acclimation and maintenance of stock fish.

The fish were placed in 200 litre aquaria with aged Sydney tap water. *P. signifer* being collected from water of variable salinity were acclimated to freshwater over seven days without mortality. The other three species were found in freshwater and therefore did not require acclimation.

A regime of two feeds per day was established. The first feed was at 0830 hours It consisted of 0.1 gram of dried commercial food (Tetramin). The second was at 1600 hours and consisted of 0.2 gram (wet weight) frozen brineshrimp. The aquaria were exposed to daylight (Australian E.S.T), supplemented by Grolux fluorescent light between 0800 and 1700 hours E.S.T. each day. After acclimation the fish were transferred to 30 litre breeding tanks maintained at 25 ± 2°C.

2.2.4 Egg production and handling.

Two pairs of *P. signifer* and *P. mellis,* one pair of *P. tenellus*, and three pairs of *P. gertrudae* were found to provide sufficient eggs for the study of embryological development in these species. *P. tenellus* spawned amongst the adventitious roots of floating *Ceratopteris* sp. The other three species favoured the leaves of Java moss, *Taxiphyllum barbieri*. Newly spawned eggs were removed from the plants and placed in petri dishes (85mm x 12mm) containing 40ml of aquarium water maintained at 24 ± 1°C. The water was partially changed at least once a day. The petri dishes were cleaned every three days.

2.2.5 Surface structure of eggs.

Eggs for scanning electron microscopy (SEM) were fixed in 3% glutaraldehyde in 0.025M phosphate buffer, pH 6.8-7.0 for 1-2 days. After fixation the eggs were rinsed several times during the next 2 days with phosphate buffer. The fixed eggs were dehydrated in an alcohol series in 10% increments from 10% to absolute ethyl alcohol at 30 minute intervals. After several changes of absolute alcohol, the eggs were dried in a critical point drier, mounted on stubs and gold coated following the procedure adapted from Cohen (1979).

All solutions (buffer, fixative and alcohols) were filtered through a 0.22μm filter to avoid contamination of the specimen with debris. The prepared eggs were then examined under a Jeol T20 scanning electron microscope. Measurements were made directly from photographs taken at known magnifications.

2.2.6 Prespawning and spawning behaviour.

Prespawning and spawning behaviour and associated colour changes were recorded for each species. Twice weekly the fish pairs from which eggs were collected were observed for spawning behaviour. A period of two hours observation was made between 0800 and 1000 hours, then at 1200 to 1400 hours and finally between 1600 and 1700 hours. In these periods the fish were observed qualitatively for pre-spawning display and spawning activity. Some observations on the frequency of spawning activity, and on the viability of eggs through the year were also noted.

2.2.7 Embryonic development.

Embryonic development was followed on ten to twenty eggs every 15 to 30 minutes for the first twelve hours and thereafter every hour to two hours until hatch. An Olympus binocular compound microscope (Model E) was used. Drawings were made of developmental stages and measurements made with a calibrated ocular micrometer.

2.2.8 Larval development.

Larvae were hatched out in white plastic containers with a diameter of 13cm. Water was aerated but not filtered. If the larvae were less than 4.5mm total length

(TL) on hatching, they were fed one drop of Liquifry No. 1 (Liquifry, Dorking, U.K.) and Tetramin E (Tetrawerke, Melle, Germany) three times daily until TL exceeded 4.5mm. Larvae greater than 4.5mm TL received only Tetramin E, supplemented with brineshrimp nauplii (*Artemia*). At 10mm TL Tetramin E was replaced with crushed Tetramin Staple Food. At 15mm TL, brineshrimp nauplii were withdrawn and replaced with frozen brineshrimp. Up to 25 larvae from each species were measured daily. Some individual larvae were measured repeatedly throughout the study. These larvae were not necessarily from the same spawning nor the same female. There was a mix of independent and non-independent measurements as individual larvae were not isolated and could not be identified. One or two specimens of each age were measured and fixed in Bouin's fixative (Culling *et al.*, 1985). Later they were cleared and stored in 70% ethanol. Measurements were made of live fish again using the Olympus compound microscope model E with a calibrated ocular micrometer.

2.3 Results.

2.3.1 Structure of the egg.

Details of the external morphology of the eggs are shown in Fig. 2.2 - 2.6 and summarised in Table 2.2. The eggs of the four species were spherical. They exhibited significant differences in the size, length and position of the chorionic filaments. The chorionic filaments were used to attach the egg to plant material. *P. signifer* produced the largest eggs (1.13-1.65mm diameter) followed by *P. gertrudae* (1.21-1.43mm) and *P. mellis* (1.26-1.31mm) with overlapping ranges, with *P. tenellus* the smallest (1.03-1.29mm diameter).

2.3.1.1 Chorionic filaments and surface structure.

The chorionic filaments were evenly distributed over the chorion in eggs of *P. signifer* and *P. mellis* (Fig. 2.2-2.3). In contrast, the eggs of *P. tenellus* had one tuft of filaments arranged in an orderly pattern at the vegetal pole and *P. gertrudae* had two tufts of filaments, one at the vegetal pole, the other at the animal pole (Fig. 2.2 and 2.3). The filaments of all eggs were elastic and extremely adhesive to plant surfaces.

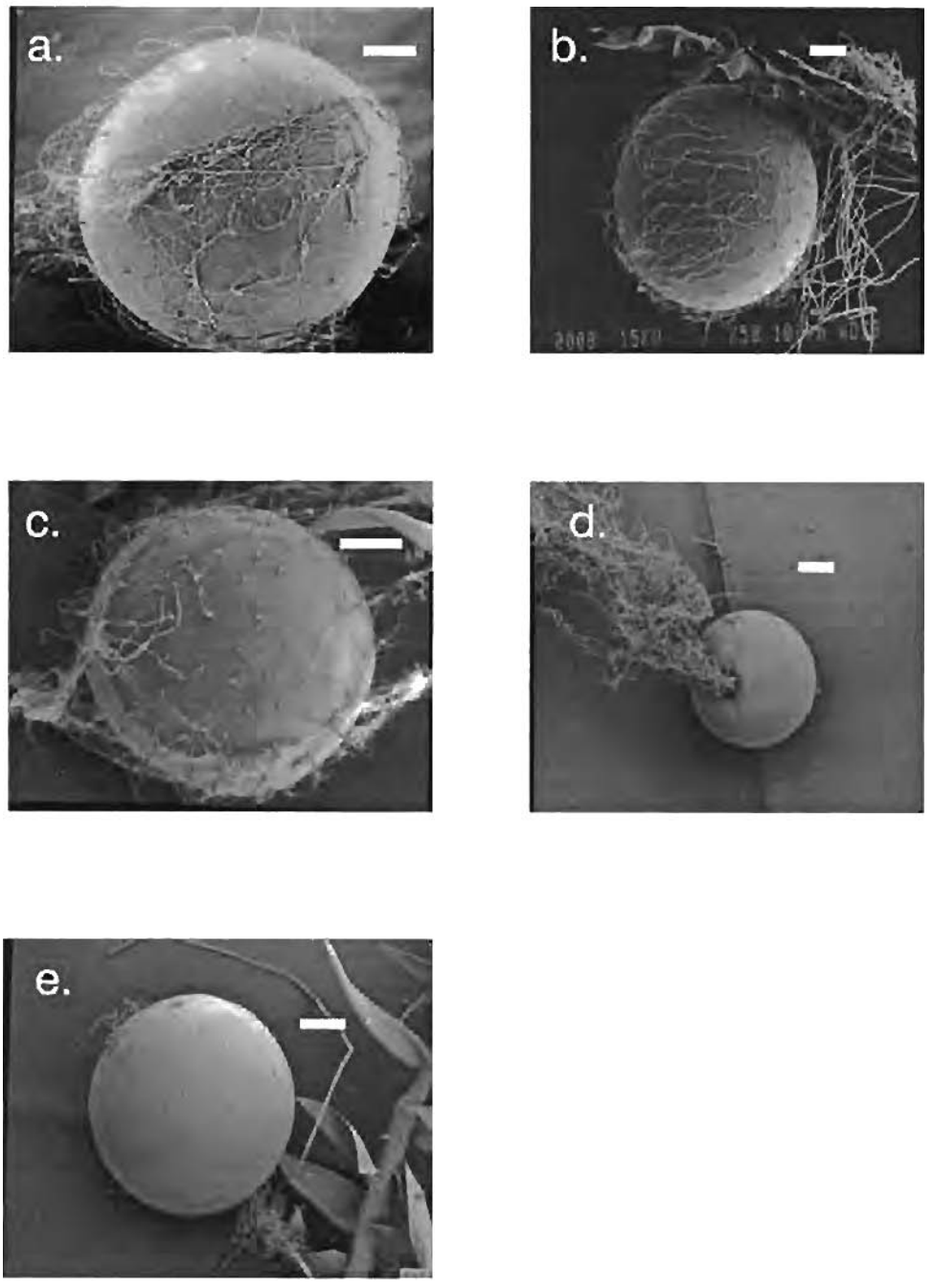


Figure 2.2 Scanning electron microscope photomicrographs of eggs of *Pseudomugil signifer* (a and b) (Narrabeen and Townsville) compared with *P. mellis* (Fraser Island) (c), *P. tenellus* (d) and *P. gertrudae* (e).
The eggs of *P. signifer* and *P. mellis* have filaments over the entire surface, whilst those of *P. tenellus* have one tuft of filaments at the vegetal pole, and *P. gertrudae* have tufts of filaments at both the animal and vegetal poles. Scale bars equal 200μm.

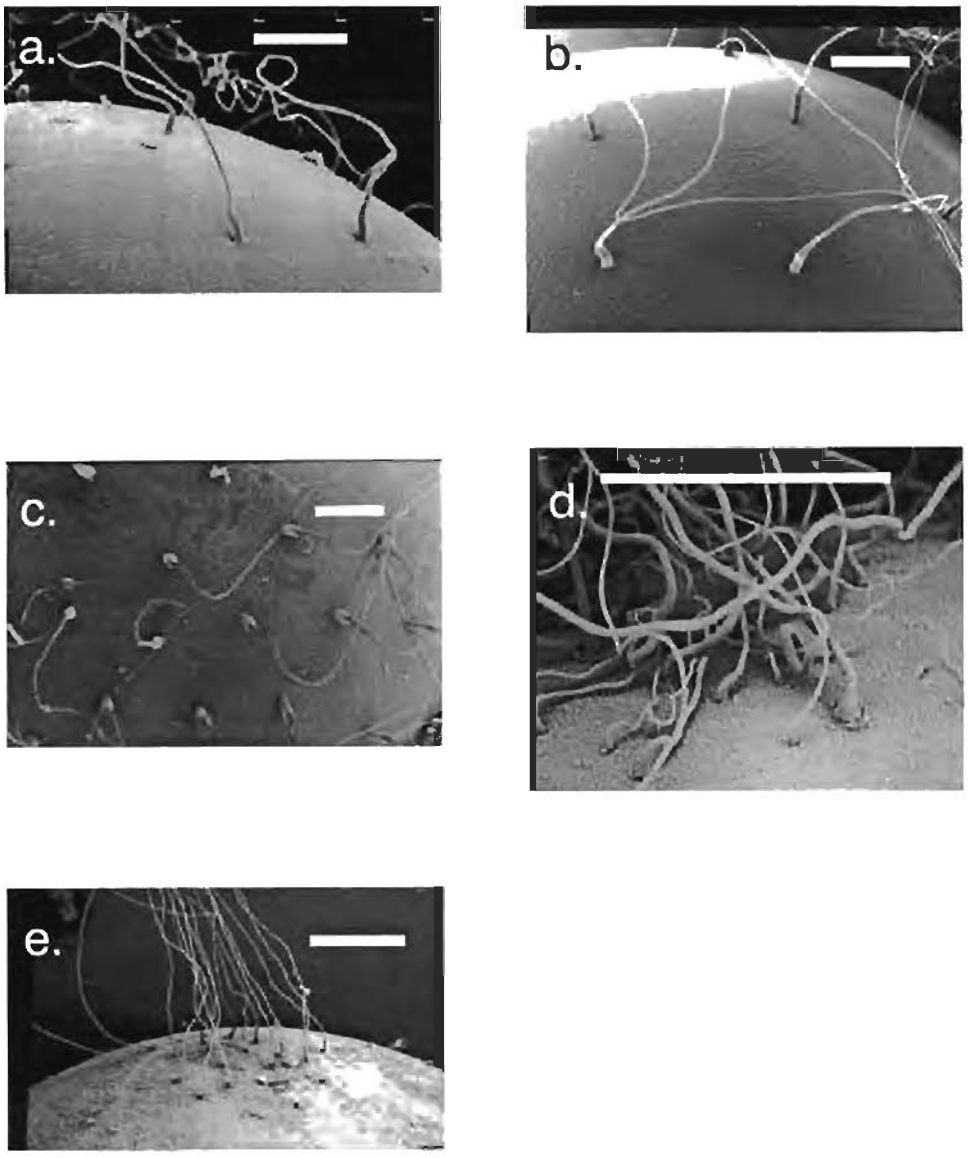


Figure 2.3 Scanning electron microscope photomicrographs of pattern of filaments on eggs of *Pseudomugil signifer* (a and b) (Narrabeen and Tuggerah) compared with *P. mellis* (c), *P. tenellus* (d) and *P. gertrudae* (e). Scale bars equal 100 μm.

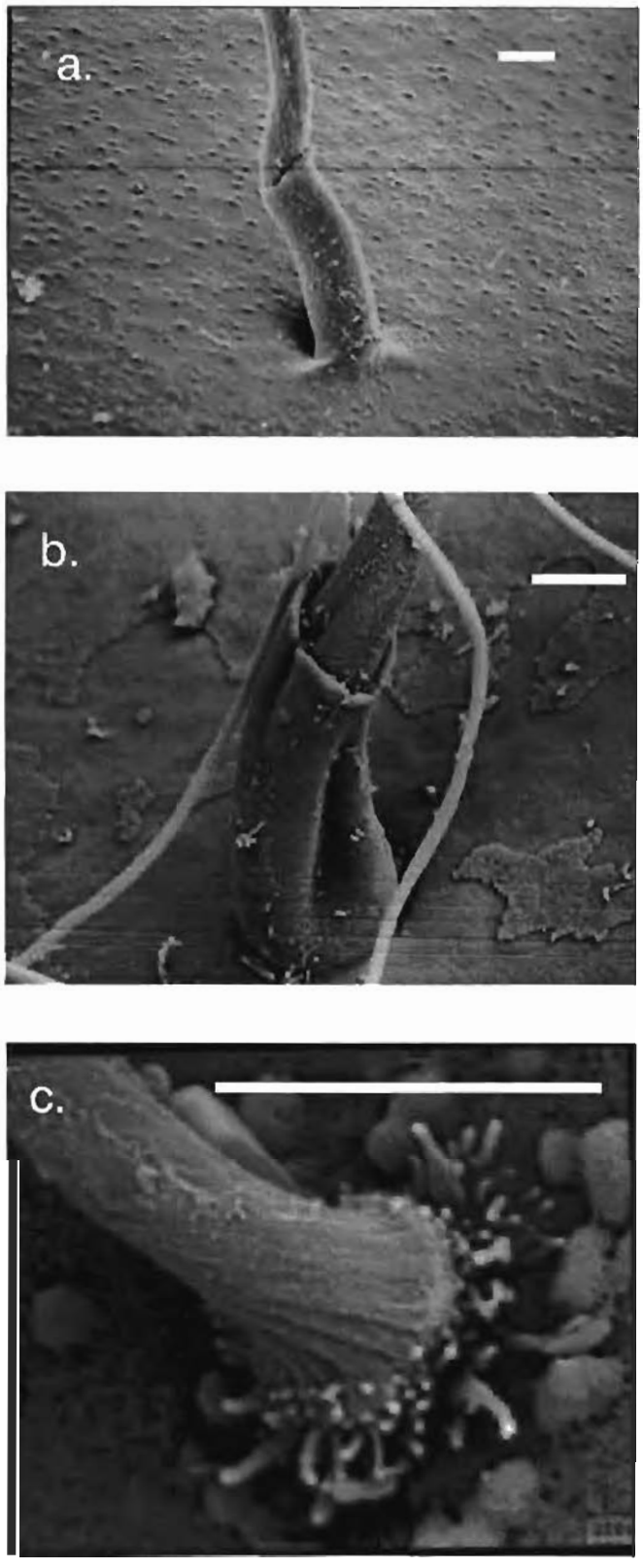


Figure 2.4 Scanning electron microscope photomicrographs of a single filament of an egg from *Pseudomugil signifer* (a and b) (Narrabeen and Smiths Lake) compared with *P. tenellus* (c). Scale bars equal 10 μm.

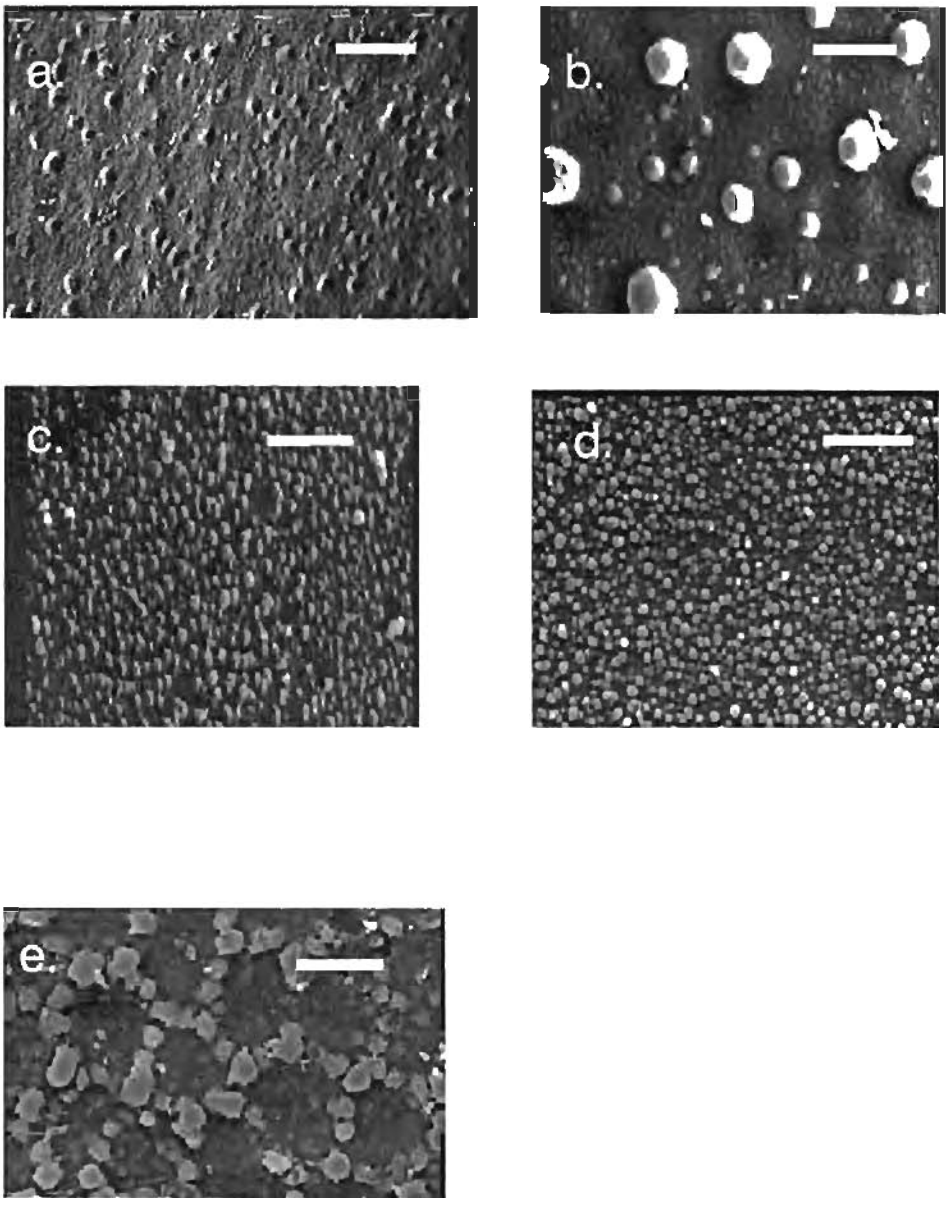


Figure 2.5 Scanning electron microscope photomicrographs of surface sculpturing on eggs of *Pseudomugil signifer* (a and b) (Narrabeen and Townsville) compared with *P. mellis* (c), *P. tenellus* (d) and *P. gertrudae* (e). The blebs on the eggs from *P. gertrudae* are in a hexagonal pattern. Scale bars equal 10 μm.

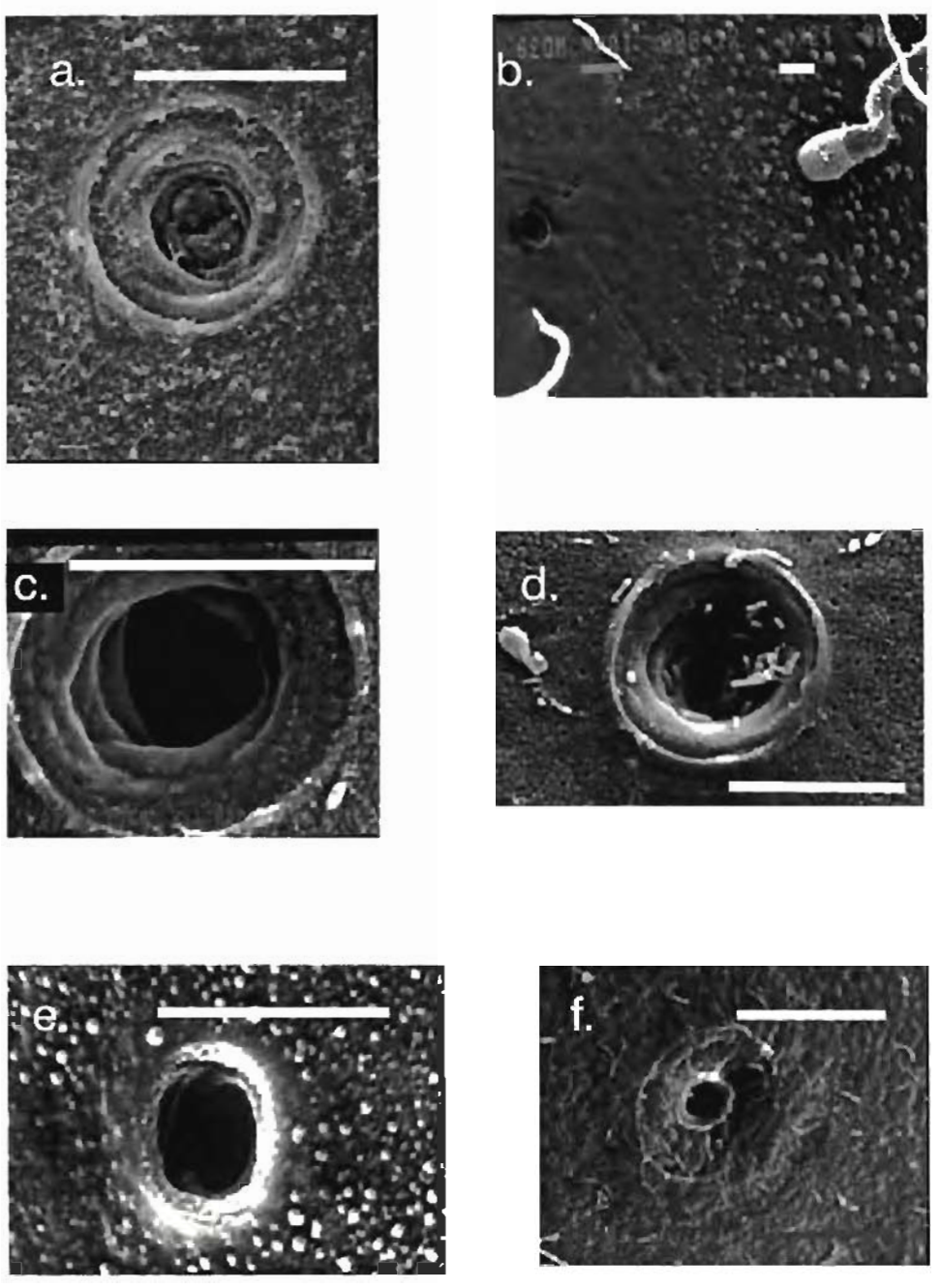


Figure 2.6 Scanning electron microscope photomicrographs of the micropyles on eggs of *Pseudomugil signifer* (a, b, c and d) (Narrabeen, Townsville, Townsville and Cairns) compared with *P. tenellus* (e) and *P. gertrudae* (f). The micropyle on the eggs of *P. tenellus* had blebs directly next to it unlike all other species observed. Scale bars equal 10 μm.

Table 2.2 Comparison of the surface filaments of the eggs of *P. signifer* with *P. mellis*, *P. tenellus* and *P. gertrudae*. Measurements were made from photographs taken with the scanning electron microscope and are in μm $\pm$ standard error. Ten measurements were made unless otherwise specified. Fil. = filament.

Species	*P. signifer* Narrabeen	*P. signifer* Smiths Lake	*P. signifer* Townsville	*P. mellis*	*P. tenellus*	*P. gertrudae*
Filaments (Tuft diameter)	Entire surface	Entire surface	Entire surface	Entire surface	One tuft (243±41, n=8)	Two tufts (359±153, n=6)
Collar length	36 ± 1.6	50 ± 2.9	31 ± 4.4	30 ± 2.8	12 ± 1.8	10 ± 1.3
Fil. Base	17 ± 0.6	17 ± 2.8	10 ± 0.6	11 ± 1.3	6 ± 0.7	5 ± 0.4
Fil. Shaft	8 ± 0.6	7 ± 0.6	5 ± 0.6	5 ± 0.6	3 ± 0.4	3 ± 0
Fil. spacing	167±18.0	176± 7.3	127±9.2	120 ±4.7	40 ±9.6	37 ±7.5

Filaments in all species have a collar at the point of attachment to the chorion. The most extensive collar was found on the egg from the southern *P. signifer* (Smiths Lake) (Fig. 2.4) which was nearly twice the length of that found in the egg of the northern *P. signifer* (Townsville). Various other differences were observed between these populations such as the diameter and length of the filament (Table 2.2).

All *P. signifer* and *P. mellis* populations examined had eggs with thicker and longer filament collars and shafts than those of *P. tenellus* and *P. gertrudae* eggs. The distance between filaments showed the same trend in that the filaments of the eggs of *P. signifer* and *P. mellis* were spaced further apart than were those of *P. tenellus* and *P. gertrudae* eggs.

The filaments of *P. mellis* were the most fragile and these eggs were the most difficult to process for SEM. It was also observed that they did not adhere to plant material as well as eggs of the other species. Extra strength was observed in the thicker *P. signifer* filaments. *P. signifer* had one size of filament in contrast to *P. mellis*, *P. tenellus* and *P. gertrudae* which had two sizes of filaments. Even when the filaments were different in size they were very similar in appearance.

The filaments of *P. tenellus* were distinct from the other species in possessing small protrusions at their base (Fig. 2.4c). They also had a slightly striated collar on

the filament which was not observed in any of the other species.

More subtle differences were observed in the texture of the egg surface and in their micropyles (an aperture through which the egg is fertilised) (Fig. 2.5 and 2.6).

Surface sculpturing, in the form of small blebs was observed on the surface of the eggs (Fig. 2.5). The blebs on the northern (Townsville) eggs of *P. signifer* were of three to four different sizes (mean diameter ± SE, n=10 was 3.2 μm ±0.3). Blebs on the eggs of *P. gertrudae* were the largest (mean diameter ± SE, n=10 was 4.5μm ± 0.3) and were arranged in a hexagonal pattern unlike any of the other species observed. The eggs of *P. mellis* and *P. tenellus* had smaller blebs (mean diameter ± SE, n=10 1.6μm ± 0.2 and 2.0μm ± 0.3 respectively).

It was not possible to obtain data on micropyles from large numbers of eggs. However, micropyle diameter measurements have been used on other species of fish eggs and have been found to be remarkably constant within species (Hawkes and Stehr, 1980; Kosmath *et al.*, 1981 and Riehl and Kock, 1989). When the eggs were mounted for scanning electron microscopy they were placed on the stub in a random orientation. The micropyle was therefore only visible on certain occasions. Micropyles were observed in *P. signifer* from Sydney, Tuggerah Lake, Lake Hiawatha (near Grafton), Townsville and Cairns, *P. tenellus* and *P. gertrudae*. Table 2.3 summarises some of their characteristics. Results were from individual eggs. *Pseudomugil signifer* (Cairns) had a larger micropyle (Fig. 2.6d) than any of the other *P. signifer* populations (Fig. 2.6a, b and c) even though they had the smallest eggs. *P. tenellus* had the smallest micropyle with a very small annulus (Fig. 2.6e). *P. tenellus* was the only species in which blebs were seen next to the micropyle, although they were reduced in size and more widely spaced near it.

2.3.2 Prespawning and spawning behaviour.

The colouration of the bodies of non-breeding fish (all species) were greyish and their fins were only lightly tinted with colour. With the onset of spawning, which occurred during daylight hours, the fins of both sexes became fully extended. Colouration in the fins and bodies of the males of each species changed throughout spawning. Such change in colours occurred only in the female *P. tenellus*, whose colouration resembled that of the male *P. tenellus*.

Table 2.3 Some characteristics of the micropyle measured from eggs after processing for SEM.

Species	Diameter of Micropyle (μm)	Diameter of hole (μm)	Diameter of unblebbed region (μm)	Other Observations
P.signifer (Narrabeen) n= 5	10.8 10.8 10.7 10.2 10.4	4.8 4.7 4.6 4.6 -	- - 906 1,650 -	No blebs near micropyle
P. signifer (Tuggerah Lakes) n= 1	10.0	3.0	None	Small holes near micropyle No holes on surface
P. signifer (Lake Hiawatha) n= 1	10.8	4.6	205	No blebs near micropyle
P. signifer (Townsville) n= 3	10.3 10.5 10.5	3.9 4.0 4.4	150 140 135	Annulus very clear. No blebs near micropyle.
P. signifer (Cairns) n= 2	13.1 15.5	5.9 5.0	Unknown Unknown	No blebs near micropyle. Small holes near micropyle.
P. tenellus n= 3	8.5 8.6 9.1	5.0 4.1 4.3	None	Blebs near micropyle
P. gertrudae n= 3	13.3 15.3 15.0	3.9 4.0 3.7	140 165	No blebs near micropyle Small holes near micropyle

Table 2.4 lists the colour changes in detail. Flashing of pectoral fins (rapid extension and retraction) was observed to a slight degree in male *P. signifer* from NSW populations but was very conspicuous in male *P. gertrudae*. Flashing was not observed in *P. signifer* from Townsville. It was not observed in the males and females of the other species. Male *P. gertrudae* also had strongly coloured pectoral fins (Table 2.4).

Table 2.4 Spawning colouration in males of four *Pseudomugil* species.

	P.signifer Smiths L. and Narrabeen	*P.signifer* Townsville	*P. mellis*	*P.tenellus*	*P.gertrudae*
First dorsal fin	White fore edge,pale orange centre grey blue under edge	Black spot at base, white fore-edge	Bright orange; fine white fore-edge, black line within margin	Bright yellow; black edge, white tip; rays black	Black spots (permanent)
Second dorsal fin	Bright orange; black edge, white tip	Pale yellow; black spot at base, black outline within white margin	Bright orange; fine white fore-edge; black line within margin	Bright yellow; black edge, white tip; rays black, white spotted	Black spots (permanent)
Anal fin	Bright orange; black edge, white tip	Pale yellow; black edge; white and black rays	Fine white fore-edge, black line within margin	Row of white spots across centre of black rays	Black spots (permanent)
Pelvic fin	Pale orange; white tip	Creamy, with thin black edge	Bright orange;white outline	Bright yellow; black edge, black rays	Creamy yellow
Pectoral fin	Black fore-edge, clear	Fine white fore-edge; strong black line inside margin	Heavy black line within white fore-edge	Colourless	Bright orange
Caudal fin	Orange; white and black edged, white tip; outer rays white	Bright yellow; dark yellow rays; tips white, with proximal black area	Bright orange; black line outlined in white on the upper & lower edge of the fin	Yellow	Black spots (permanent)
Body colour	Bronze	No colour change	Honey colour in caudal region	Dark sulphurous yellow	Black spots (permanent)
Disting-uishing body markings	Mid-lateral line of glowing spots	Mid-lateral line of glowing dots; body purple in caudal area	Mid-lateral line of glowing dots	Mid-lateral, white and gold iridescent spots	Small black dots (permanent)

In all four species, the prespawning period varied from a few minutes to several hours. The male chased the female rapidly, circling closely without nudging or other direct contact. This continued until spawning occurred. This pre-spawning behaviour resumed after spawning on many occasions, although subsequent spawning was not

observed on the same day. Males of *P. mellis* were the most persistent in that the male continued to chase the females in a more aggressive manner and for a much longer period than in the other species.

The species differed in the location of spawning relative to the macrophyte stands. *P. signifer* and *P. mellis* spawned low on the outside of the Java moss clumps near the substrate, whereas *P. gertrudae* entered the Java moss clumps midway from the substrate before spawning. *P. tenellus* preferred to spawn at the water surface in the roots and fronds of floating plants (*Ceratopteris* sp.).

Spawning was not inhibited by the presence of more than one pair of the same species in the same tank. Up to 10 pairs of *P. gertrudae* have been maintained in a tank with no interruption to spawning.

Spawning activity and the viability of the eggs were at a maximum during the summer months, November-February. Both gradually declined through March-April. Spawning ceased during the winter months in all species, except *P. gertrudae*. In September-October spawning activity and egg viability gradually increased reaching their summer levels by November. *P. gertrudae* appeared to be the most prolific species. Eggs from this species were obtained each month, but the eggs were usually non-viable during the winter months.

Spawning did not follow a daily pattern, but continued over several days followed by a period of inactivity. *P. tenellus*, in particular, showed periods of 1-2 weeks inactivity during the summer months. If spawning ceased for a short period during the summer months a water change, which frequently lowered the temperature, or introduction of new plants sometimes induced spawning in all species. *P. tenellus* had the highest fecundity (releasing the largest number of eggs in one day) compared with the other species.

2.3.3 Embryonic development.

The pattern of development, was essentially the same in all species. It is presented diagrammatically in Fig. 2.7 and the time course of developmental events is illustrated in Fig. 2.8.

Cell division in all species was meroblastic, and occurred at the animal pole, the opposite end of the egg from the filaments in *P. tenellus*.

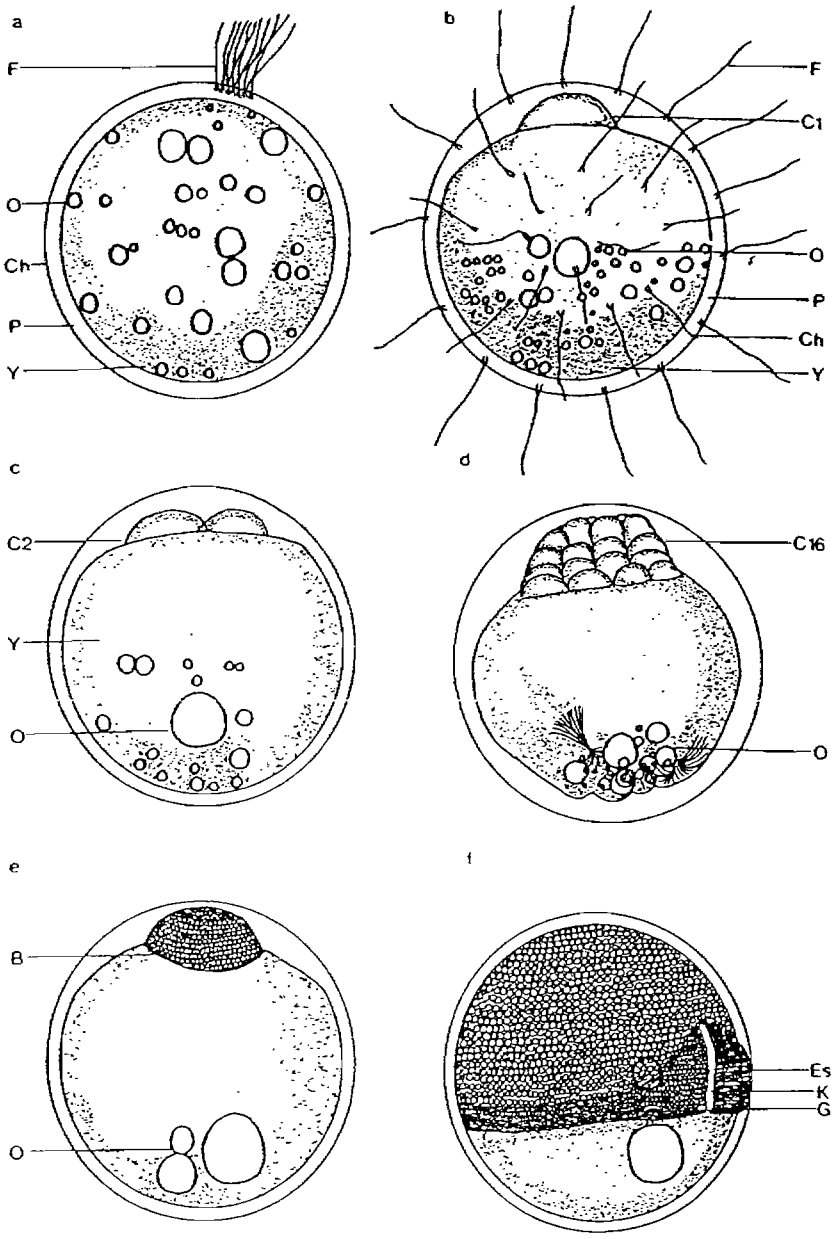


Figure 2.7. Embryonic development of *P. signifer, P. mellis, P. tenellus* and *P. gertrudae.* Sketches showing 13 stages are given as hereunder.

a) Fertilisation. Filaments as for *P. tenellus.*

b) One cell stage. Filaments as in *P. signifer.*

c) Two cell stage.

d) Sixteen cell stage. Distortion of yolk as in *P. tenellus.*

e) Successive cleavages, increase in the number of cells with a reduction in cell size.

f) Gastrula has expanded to cover half the yolk.

Various features are indicated by symbols as follows:-

B -Blastoderm, Br - Brain, C1 - one cell, C2 - two cells, C16 - sixteen cells, Ch - Chorion, E - Eye, Es - Embryonic shield, F - Filaments, G - Germ ring, K - Keel of central nervous system, KV - Kupffer's vesicle, L - Lens, M - Melanophores, O - Oil droplets, Op - Optic vesicle, Ot - Otic vesicle, P - Perivitelline space, Pe - Pericardial cavity, Pf - Pectoral fin, S - Somites, T - Tail, U - Urinary bladder, Y - Yolk.

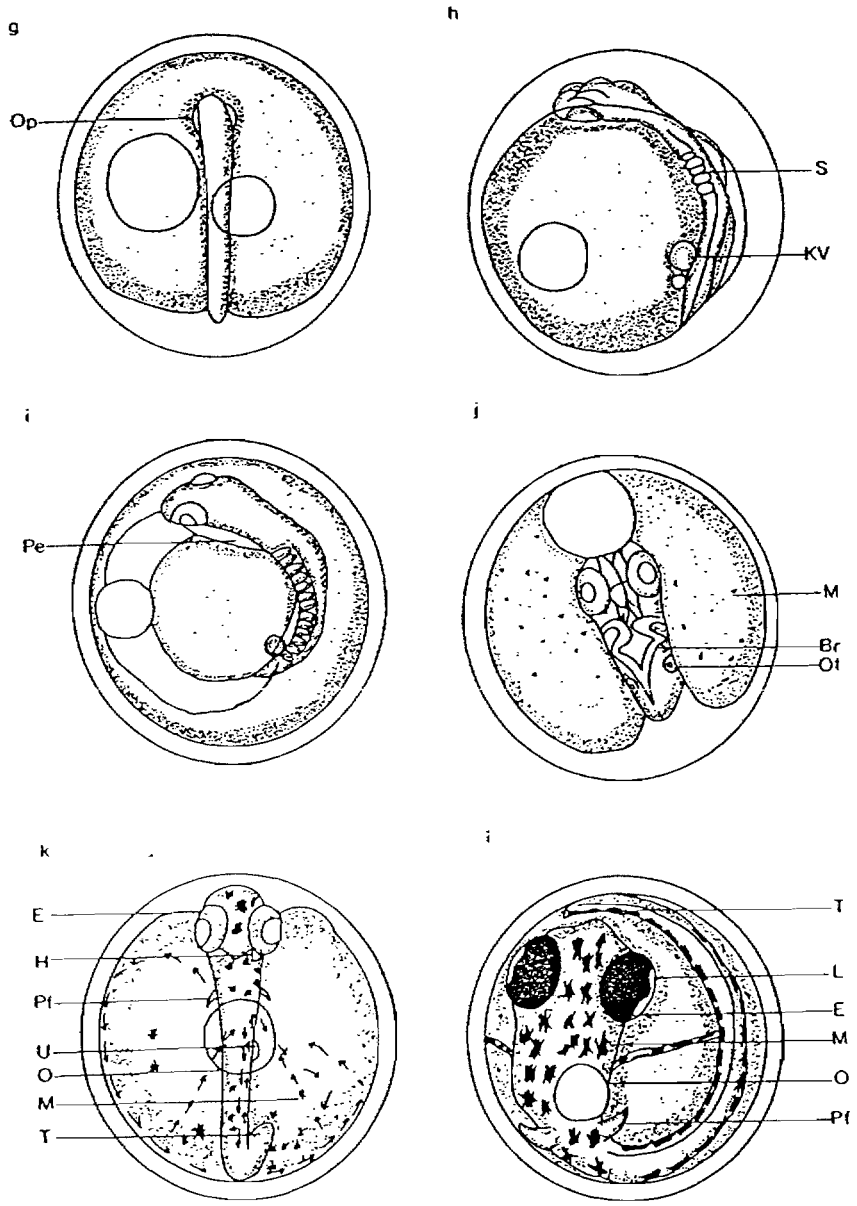


Figure 2.7 Continued

g) Optic vesicles present.

h) Several caudal somites and Kupffer's vesicle present.

i) Pericardial cavity and optic cup present.

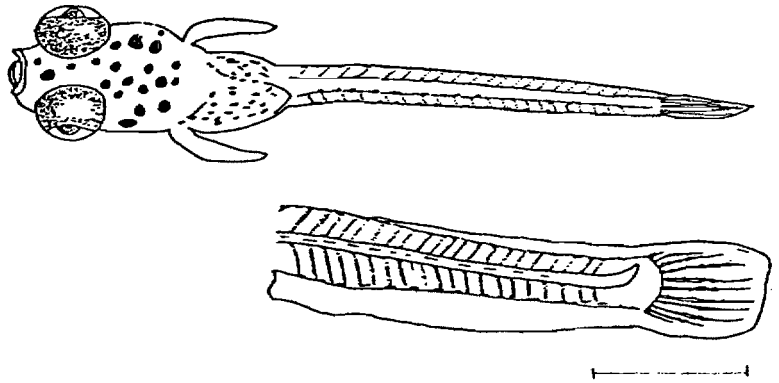
j) Otic vesicles, brain fairly well defined. Light melanophore pattern on yolk sac of *P. gertrudae* and *P. tenellus* only.

k) Heart present, flow of blood indicated by arrows, urinary bladder and pectoral fins.

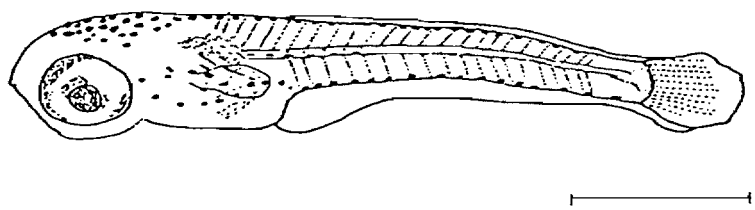
l) Eye pigmented and lens formed.

(m)

(i)



(ii)



(iii)

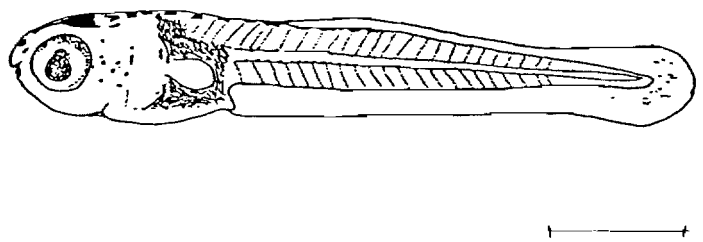
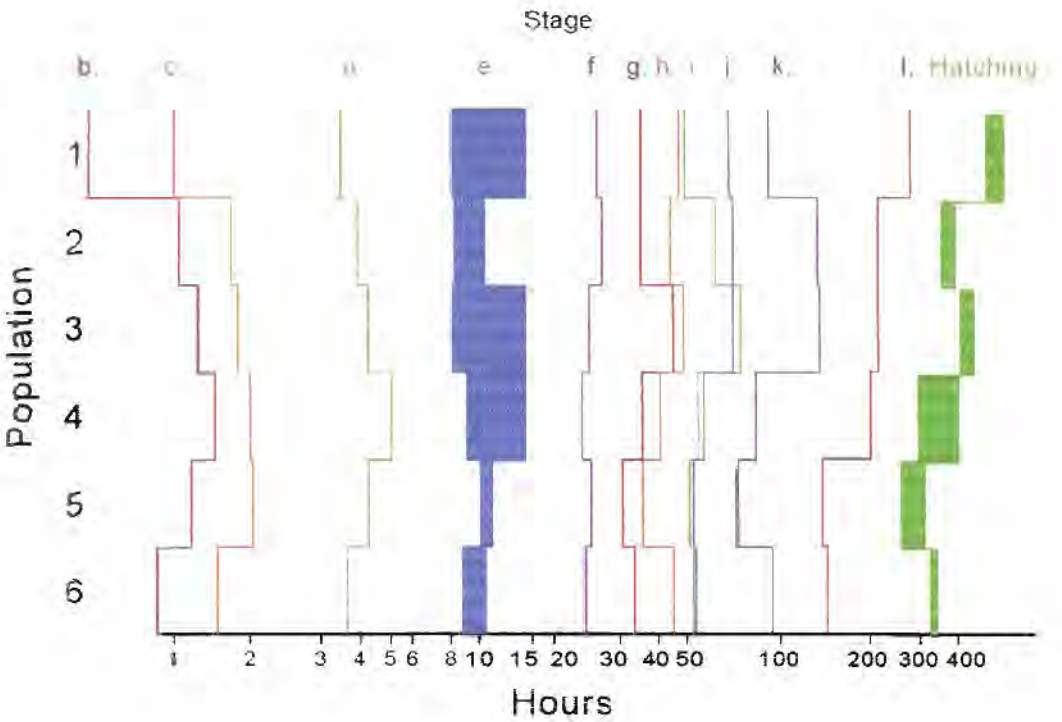


Figure 2.7 continued
m) Newly hatched *P. signifer* (i), *P. tenellus* (ii) and *P. gertrudae* (iii) (scale bars equal 1mm).



1. *Pseudomugil signifer* (Narrabeen, NSW).
2. *P. signifer* (Smiths Lake, NSW).
3. *P. signifer* (Townsville, Qld).
4. *P. mellis*.
5. *P. tenellus*.
6. *P. gertrudae*.

Figure 2.8. Embryological development (depicting different stages) of *Pseudomugil signifer*, *P. mellis*, *P. tenellus* and *P. gertrudae* from spawning to hatching at a controlled temperature of 24±1°C. Five to ten eggs were examined from each species. Stages depicted here correspond to those in Figure 2.7. Fertilisation in all species was effected within two seconds. Time is presented on a log scale.

Table 2.5 summarises and compares some characteristics of the eggs of *P. signifer*, *P. mellis*, *P. tenellus* and *P. gertrudae*.

Table 2.5. Comparison of the eggs of *P. signifer* with *P. mellis*, *P. tenellus* and *P. gertrudae* (ranges were measured in ten to twenty eggs).

Parameter range	*P. signifer* Narrabeen	*P. signifer* Smiths Lake	*P. signifer* Townsville	*P.mellis*	*P.tenellus*	*P.gertrudae*
No of oil droplets at fertilisation	40-80	40-50	50-80	90-100	>100	30-40
Size of oil droplets (mm)	0.015-0.460	0.005-0.170	0.005-0.260	0.005-0.240	0.005-0.180	0.01-0.170
Perivitelline space (mm)	0.03-0.20	0.10	0.05-0.13	0.05-0.18	0.05	0.10
Diameter of egg (mm)	1.50-1.60	1.55-1.65	1.13-1.52	1.26-1.31	1.03-1.29	1.21-1.43
No of eggs spawned	2-7	2-7	2-8	1-4	3-15	3-6

In the eggs of *P. gertrudae* the filament tufts occurred both over the animal pole and at the vegetal pole. Shortly after fertilisation, which occurred within seconds, numerous oil droplets appeared in the yolk of all species (Fig. 2.7a). These droplets varied in size and number between species (Table 2.5) and moved to the vegetal pole during the first four cleavages (Fig. 2.7c). As development progressed, the number of oil droplets decreased, and by the time a pericardial cavity appeared only one to three much larger droplets remained (Fig. 2.7i), suggesting that the smaller droplets may have coalesced to form the larger droplets. In the eggs of *P. tenellus* coalescence of droplets following the 16 cell stage greatly distorted the yolk (Fig. 2.7d). This did not occur in the eggs of other species.

At a temperature of 24±1°C the developmental rate up to the blastoderm stages was similar in all species; although early cell division in *P. mellis* was slower (Fig. 2.7d). After the blastoderm stage, eggs of *P. signifer* from Narrabeen and Townsville took longer to hatch (21-24 and 16.5-19 days respectively) than those from Smiths Lake (14-15.8 days), even though the early development rates were fairly comparable (Narrabeen 8-14, Smiths Lake 8-10 hours and Townsville 8-14

hours, to blastoderm stage, Fig. 2.7e).

Unlike the other species, the eggs of *P. signifer* did not develop melanophores on the yolk sac.

The duration of embryonic development varied between species, the slowest rate was for *P. signifer* (fertilisation to hatching 14-24 days), *P. mellis* and *P. gertrudae* developed a little faster (fertilisation to hatching interval 12-13.5 days) and the most rapid development occurred in *P. tenellus* (fertilisation to hatching interval 10-12 days) at a temperature of 24 ± 1°C.

2.3.3.1 Hatching.

Pectoral fins commenced to move rapidly with mouth opening and shutting leading to hatching.

2.3.4 Larval development.

Developmental features of the larvae were related to length rather than age in *Pseudomugil*. For this reason information was accumulated on development by their length, not their age. Table 2.6 details the lengths of larvae at which major features developed.

2.3.4.1 Finfold.

P. signifer (Townsville): the dorsal finfold commenced at the fourth post-anal myomere.

P. signifer (Cairns): the dorsal finfold commenced at the second preanal myomere.

P. signifer (Narrabeen): the dorsal finfold commenced on the third to fourth postanal myomere.

P. tenellus: the dorso-ventral fin fold extended from the first post-anal myomere dorsally to the anus ventrally. There was no preanal finfold.

P. gertrudae: the dorso-ventral finfold commenced at the second post-anal myomere.

P. signifer and *P. tenellus* had a very developed caudal fin on hatching unlike *P. gertrudae* (See Fig. 2.7).

Table 2.6. Minimum lengths of larvae at which some major features were first observed (mm).

	P. signifer Narrabeen n=17	*P. signifer* L. Hiawatha n=6	*P. signifer* Townsville n=28	*P. signifer* Cairns n=6	*P. tenellus* n=7	*P. gertrudae* n=12
Pectoral fin	Present at hatch 5.5	Present at hatch -	Present at hatch 5.0	Present at hatch 5.4	Present at hatch 4.1	Present at hatch 4.5
Rays & spines	-	7.8	8.5	6.6	4.3	7.6
Branched rays	-	-	16.5	17.9	15.4	11.2
Caudal fin	Present at hatch 5.5	Present at hatch -	Present at hatch 5.0	Present at hatch 5.5	Present at hatch 4.1	Present at hatch 5.3
First rays	5.6	-	5.0	5.5	4.1	5.3
All rays present	12.5	12.8	7.3	10.0	9.8	9.0
Branched rays	17	16.7	16.5	16.2	14.0	13.0
Fin forked	13.4	12.8	13.5	11.8	12.8	11.0
Pelvic fin	9	10.0	9.0	10.1	7.4	9.0
Rays	-	10.1	10.4	10.1	9.0	9.3
Branched rays	13.25	-	18	17.9	15.9	16.0
Anal fin	9	7.6	6.7	8.0	5.3	6.6
Rays	12.5	10	7.3	10	8.9	8.9
Branched rays	17	-	17.9	17.9	15.4	16.0
Second dorsal	9.8	8.0	7.9	8.2	6.8	7.5
Rays	-	10.0	9.6	9.6	8.4	9.2
Branched rays	17	-	18.0	19.5	15.4	15.0
First dorsal	-	12.8	12.0	11.6	9.2	9.0
Rays	17	-	13.0	12.9	10.7	9.4
First scales	12	-	11.3	12.4	9.8	9.4
Squamation	18	-	18.0	17.1	16.0	12.5
Finfold disappears	15	-	16.8	15.8	12.9	-

2.3.4.2 Fins.

With the exception of the first dorsal fin, the fins appeared at a similar total length in all three species. The first dorsal fin developed when *P. signifer* was larger than *P. tenellus* or *P. gertrudae*. The species could be separated on the number of second dorsal fin rays developed; *P. tenellus* had six rays, *P. gertrudae* had seven or eight rays and *P. signifer* from L. Hiawatha had eight or nine rays, while *P. signifer* from Cairns had nine to eleven rays.

2.3.4.3 Myomeres.

It was not possible to count the preanal myomeres on the ventral surface of the body in the larvae as their bodies were very darkly pigmented. Less postanal myomeres were counted in *P. tenellus* (17-19) than in *P. gertrudae* (18-20) or *P. signifer.*

2.3.4.4 Melanophores.

All species observed were pigmented with melanophores, *P. gertrudae* having the heaviest pigmentation on the body. Upon hatching *P. gertrudae* had a well defined melanophore pattern on the mouthparts unlike *P. tenellus* and *P. signifer.* Caudal rays were outlined by melanophores in *P. tenellus* and *P. signifer* upon hatching but not in *P. gertrudae. P. gertrudae* had the greatest number of melanophores on their pectoral fins. *P. tenellus* had a lesser number, and *P. signifer* had very few, or, in the case of the Townsville population, none.

2.3.4.5 Squamation.

Squamation occurred at a lesser length in *P. gertrudae* (9.4 mm) and *P. tenellus* (9.5 mm) than in *P. signifer* (10.5 - 12.4 mm).

2.3.4.6 Secondary sexual characteristics.

By 21 mm TL secondary sexual characteristics were apparent in the form of colouring in *P. tenellus.* By 16mm TL the sexes could be identified by fin formation and colour in *P. gertrudae.* By 18mm TL the sexes could be identified by fin formation and colour in *P. signifer* from Narrabeen, Smiths Lake and Townsville. Male *P. signifer* from Cairns could be distinguished from females at a TL of 26 mm.

The secondary sex characteristics were evident from the age of six months to a year dependent on the length of the fish.

2.4 Discussion.

2.4.1 Egg characteristics.

Characteristics associated with the chorion have been useful in identifying fish eggs and have been shown to be highly adapted to the environmental conditions under which an embryo developed (Matarese and Sandknop, 1984). White, *et al.* (1984) examined a large number of atheriniform eggs and found that almost all had filaments. In Australia, atherinids, melanotaeniids and the pseudomugilids fall into this group of fish.

Lonning (1972) suggested that similarities in the chorions of distantly related or unrelated species may be due to similarities in reproductive physiology and behaviour. Since this dissertation was concerned with reproductive characteristics of *P. signifer* it was relevant to study the chorion of eggs of *P. signifer* in detail. There are conservation implications for other *Pseudomugil* species, therefore it was of great interest to detail observations on the chorion of other *Pseudomugil* species. The chorions of the four species of *Pseudomugil* studied were undoubtedly similar due to their functional similarities. Eggs of the northern populations of *P. signifer* (Townsville) had blebs on their surface of three to four different sizes whilst those of the southern populations (Narrabeen, Smiths Lake and Tuggerah) were less sculptured. Sculpturing has been suggested to be of use in increasing surface area (Robertson, 1981). The eggs of *Pseudomugil* do not have extensive sculpturing but do form predominant blebs in the more northerly groups. These would increase the chorionic surface area and could be useful in promoting oxygen uptake in warmer waters where the saturation level of dissolved oxygen is lower. It may be interesting to investigate the sculpturing of the eggs further, as differences in the chorion structure can be related to their environment (Lonning, 1972). The observed differences in fine structure may be instructive and taxonomically useful within the genus. In this study it was determined that the Australian species of *Pseudomugil* examined had chorionic filaments. Anderson (1974) suggested that a possible function of the chorionic fibrils was to help the egg adhere to the substratum. The majority of *Pseudomugil* eggs observed were found attached to plant material by their filaments. There were clear morphological differences between the chorions of

eggs of the four species of *Pseudomugil.* Differences were observed between species of *Pseudomugil* in the structure of the micropyle. It had been suggested that the surface structure, in particular the micropyle may be used as a taxonomic tool (Riehl, 1979), although it should not be viewed in isolation.

2.4.2 Is a saline environment necessary for breeding?

Ivantsoff (1980) stated that a salty environment was essential for breeding of *P. signifer.* However, it was found in the current study that all four *Pseudomugil* species spawned in freshwater. *P. signifer* occurred over a broader range of salinities than the other species, which preferred freshwater (Merrick and Schmida, 1984). The ability to breed in both fresh and saline conditions, as found in this study, enabled *P. signifer* to exploit a wider range of habitats than the other species.

2.4.3 Review of *Pseudomugil* species.

On the basis of observations on four Australian *Pseudomugil* species they may be divided into two groups. One group comprised *P. signifer* and *P. mellis* and the other *P. gertrudae* and *P. tenellus.* The chorionic filaments covered the surface of the chorion in *P. signifer* and *P.mellis,* whereas in *P. tenellus* the chorionic filaments occurred in a single tuft at the vegetal pole and in *P. gertrudae* two tufts (one at each pole) were observed. *P. tenellus* and *P. gertrudae* shared a similar geographic distribution. *P. tenellus* was found in the Alligator Rivers system, within the geographic distribution of *P. gertrudae,* which was found across the Northern Territory into Cape York (Merrick and Schmida, 1984). *P. mellis* occurred in the vicinity of Noosa Heads and on Fraser Island in Queensland within the range of *P. signifer,* which was found on the east coast of Australia from Ulladulla, N.S.W. to Cooktown in Queensland. In an aquarium situation, *P. signifer* and *P. mellis* selected similar breeding sites close to the substrate on the outside of Java moss clumps. Grouping *P. signifer* and *P. mellis* is supported by observations of Allen and Ivantsoff (1982) who reported that the two species were similar in meristics, squamation and appearance. *P. signifer* and *P. mellis* had similar body shape and colour.

The members of the two groups of *Pseudomugil* species also showed some different features. *P. tenellus* and *P. gertrudae* did not share similar external

appearance. Embryonic development was more rapid in *P. tenellus* (fertilisation to hatching interval, 10-12 days) than in *P. gertrudae* (fertilisation to hatching interval, 13-13.5 days). The number of oil droplets was much greater in *P. tenellus* (> 100 per egg), than in *P. gertrudae* (20-40 per egg). Coalescence of droplets after the 16-cell stage greatly distorted the yolk in *P. tenellus*. This did not occur in *P. gertrudae* nor in the other two species. These oil droplets were thought to play some part in the supply of nourishment to the developing embryo (Russell, 1976) and also gave the egg buoyancy and stability (Munro, 1942). *P. tenellus* differed from *P. gertrudae* in the selection of breeding sites. *P. tenellus* preferred to spawn at the surface of the aquarium amongst adventitious roots of floating plants. In contrast *P. gertrudae* entered the Java moss in mid water.

P. signifer and *P. mellis* showed fewer differences, the most conspicuous being the absence of melanophores on the developing egg yolk in *P. signifer*. Melanophores were well developed on *P. mellis* egg yolks and in the other two species.

On the basis of the similarities and differences described here it was concluded that *P. tenellus* was more distinct from *P. gertrudae* than *P. mellis* was from *P. signifer*. The selection of breeding sites may act as an isolating mechanism in the overlapping species of *P. tenellus* (prefers surface) and *P. gertrudae* (prefers Java moss). But this would not appear to be so for the overlapping species *P. signifer* and *P. mellis*, as choice of breeding sites was similar for the two species.

P. signifer populations from Townsville and those from Smiths Lake and Narrabeen, Sydney had similar egg surface morphology and a similar breeding and spawning ritual, supporting the conclusion of Hadfield *et al.* (1979) that the northern and southern populations were of the same species.

The eggs of the Narrabeen population developed to hatching at a slower rate (16.3-24.3 days) than the Townsville population (16.5-19 days). The Smiths Lake population were the quickest to hatch (14-15.8 days). These differences in populations do not seem to reflect a clinal distribution in fertilisation-hatching intervals, rather a discontinuous variation. The eggs of the Narrabeen population developed rapidly up to the stage of establishment of the circulatory system (stage k, Fig. 2.8), being retarded only at the later stages.

The male *P. signifer* from Townsville differed slightly from those of the southern population in their breeding colouration and in the possession of a filamentous extension of the first dorsal fin. These features suggested that divergence was occurring between the two populations at the northern and southern extremes of the range of *P. signifer.*

In general the pattern of development of the four *Pseudomugil* species was similar with fertilisation to hatching intervals from 10-24 days. In contrast fertilisation to hatching intervals in *Melanotaenia nigrans*, *M. duboulayi*, *M. fluviatilis*, *M. splendida inornata*, *M. australis* and *M. splendida splendida* (5-6 days Crowley, 1983), were less than half that of the *Pseudomugil* species. This was at least partially explained by the observation that the *Pseudomugil* species were more developed prior to hatching. They were much larger and the caudal fin was more developed than in the Melanotaeniidae described by Crowley (1983).

Eggs of *Craterocephalus stercusmuscarum* hatched in a comparable time (13 days) to that of the four *Pseudomugil* species but the eggs of *Craterocephalus sp. nov.* hatched in only six days (Ivantsoff *et al.*, 1988). Both the eggs and newly hatched larvae of *Craterocephalus sp. nov.* were smaller than those of *C. stercusmuscarum.* In both the *Pseudomugil* and *Craterocephalus* species mentioned above, smaller eggs appeared to hatch earlier which may reflect differences in food reserves due to size. Blaxter (1984) found that tank reared herring were shorter and fatter compared to wild caught specimens. This may also reflect changes due to rearing in culture. Succeeding generations in culture spawn smaller eggs than the wild caught first generation. Reasons for this may include an immature breeding group or suboptimal culture conditions (Kamler, 1992).

Egg diameter measurements should be viewed with caution as it has been observed that as a fish ages its eggs increase in diameter (Bagenal, 1971; pers. obs.). In addition, it is known that fish belonging to the same species can produce eggs with different diameters (Bagenal, 1971) and diameters of eggs of aquarium reared fish may not be entirely reproducible (Kamler,1992). However, the comparisons are legitimate in that they were made under identical conditions and at the same time period.

Egg sizes can vary over the breeding season. Reduction in egg size can occur in

populations of seasonally spawning fish as the younger fish spawn after the older ones resulting in smaller egg size at the end of the breeding season (Bagenal, 1971; Kato, 1975).

It was observed that eggs of *Pseudomugil* species with larger diameters gave rise to larvae that were larger. Greater size of larvae from larger eggs than those from smaller eggs has been previously reported (Kryzhanovskij, 1940). As observed in *Melanotaenia* sp. by Crowley (1983) it was not the age but the length of the fish which determined when major features occurred. If, for some environmental reason, newly hatched larvae are prevented from growing at a normal rate (by, for example, reducing the volume of available water, or as a result of a pollutant, or by hiding away from predators (e.g. *G. holbrooki*) and not getting adequate food) then fin development and squamation will not occur quickly, which could be detrimental to the fish. The developmental morphology of the three species appeared to be relatively similar. There was little proportional difference in the development of the *Pseudomugil* larvae studied, as found by Crowley (1983) in *Melanotaenia*. However, *P. gertrudae* was less advanced upon hatching than the other species. Small differences were sufficient to assign the fish to particular species. *P. gertrudae* had larger pectoral fins than *P. signifer* or *P. tenellus* at the same total length. The pectoral fins in *P. gertrudae* are perhaps used more than the other two species in their courting display.

Eggs of *Pseudomugil* species of this study took a longer period to hatch, were much larger (when comparisons were made of total length), and were more developed when compared with the *Melanotaenia* species observed by Crowley (1983). Since *Pseudomugil* has been placed with the family Melanotaeniidae (Merrick and Schmida, 1984) the observed differences could well be significant. Allen (1980) also placed the genus *Pseudomugil* with the Melanotaeniidae. The details of egg surface morphology, the spawning behaviour, and embryonic development observed here differed somewhat from those observed in *Melanotaenia* by Crowley (1983). The *Pseudomugil* species are smaller fish and produce smaller numbers of larger eggs than those seen in *Melanotaenia*.

In conclusion, it was found that the egg surface characteristics of *P. signifer* did show some variation between the northern and southern populations but when these

differences were compared with other species of *Pseudomugil* they were not as pronounced. The differences observed could be ascribed to clinal variation as described by Hadfield *et al.* (1979). However, the micropyle and surface structure of *P. signifer* eggs from Cairns did not conform with those of other *P. signifer* populations in agreement with Hadfield *et. al'*.s (1979) electrophoretic data from which they concluded that the Cairns group might be isolated and undergoing speciation. Coupled with their differences in spawning behaviour these results may add weight to this proposition. The group may be becoming behaviourally as well as geographically isolated.

Differences were observed between the breeding behaviour and embryology of *P. signifer* and the other three *Pseudomugil* species. However, they do not differ from each other so much as they differ from members of the Melanotaeniidae, which have a much shorter embryological period. Said (1985) has placed *Pseudomugil* species in their own family, Pseudomugilidae, separate from the Melanotaeniidae, on the basis of osteological investigations of some Australian atherinoids. The present study distinguished the reproductive biology of *Pseudomugil* species from melanotaeniids and supported Said's view.

This section of work provided background information on the normal breeding behaviour and embryology of four Australian *Pseudomugil* species. Taxonomic implications were discussed in relation to the current taxonomic understanding of the genus. It is necessary to have knowledge of the fundamental breeding biology and taxonomy of a species before any interpretation of factors that might affect their reproduction can be made. Part of the aim of this thesis was also to look at the impact of *G. holbrooki* on *P. signifer*. Many studies have been carried out on the reproductive physiology and life history of *G. holbrooki* (Krumholtz, 1948; Innes, 1966; Trendall, 1982; Trendall, 1983; Pen, 1990; Meffe and Snelson, 1989; Brown-Peterson and Peterson, 1990; Pen and Potter, 1991; Cech *et al.*, 1992 and Vondracek *et al.*, 1988) but such data were lacking on *P. signifer*. To provide additional information on the reproductive biology of *P. signifer* the following section of research was designed firstly to determine whether *P. signifer* has a seasonal reproductive cycle and secondly to evaluate some physical and chemical environmental characteristics that may influence their reproduction.

Chapter 3.

Field study of reproductive activity in *P. signifer.*

3.1 Introduction.

As mentioned previously *P. signifer* is the most widely distributed species of *Pseudomugil* within Australia. Its geographic range extends along the east coast from Ulladulla, N.S.W. (300 km south of Sydney) to the Cooktown area in Queensland. It is also found on offshore islands such as Fraser and Lizard Islands (Merrick and Schmida, 1984).

P. signifer's habitat ranges from marine to freshwater. It is a hardy fish that has been kept by aquarists. Maintenance, breeding and embryology studies have been published (Merrick and Schmida, 1984; Semple, 1986; Howe, 1987) and have been discussed in Chapters 1 and 2 of this thesis. It was initially thought that *P. signifer* would breed only under saline conditions (Ivantsoff, 1980) but Howe (1987) found that they successfully bred in freshwater.

Very little is known about the reproductive biology of *P. signifer* in relation to their environment. Most fish are seasonal breeders, controlled by internal rhythms that are aligned with environmental factors (Bye, 1984). Bye (1984) points out that insufficient species have been studied to allow generalisations to be made on the stimuli that cause the fish to breed. This study was undertaken to determine whether *P. signifer* has a seasonal reproductive cycle. Two sites were selected to determine whether there were any differences between sites in their reproductive pattern. Factors contributing to their sexual maturity (or cycle) have not previously been studied in detail.

It was also considered of importance to determine whether *P. signifer's* gonad development occurs in the same manner in captivity as in the wild. If gonad development is similar in captive populations to that of the wild, these fish could prove to be useful for further reproductive studies in the laboratory.

In the present study an investigation was made of the seasonal cycles of two major physico-chemical conditions, salinity and temperature and the patterns of gonadal maturity in *Pseudomugil signifer* over that period. In addition, at the commencement of the experiment, groups of *P. signifer* derived from both study sites were

transferred to aquaria and maintained for one year under the conditions of culture described in Chapter 2, in order to compare the reproductive performance of the captive and wild groups.

For wild groups, gonadal structure in *P. signifer* was observed monthly in order to examine seasonal patterns of oogenesis and spermatogenesis. Such information was then correlated with data on the breeding behaviour of the fish, derived from the tank experiments reported in the previous Chapter (Chapter 2). In addition to increasing an understanding of the ecology of this species information obtained from this study could be extrapolated to other Australian *Pseudomugil* species (in particular *P. mellis* and *S. vermeilipinnis*), that appear to be even more vulnerable than *P. signifer.* In this study, emphasis was placed mostly on the ovarian development of *P. signifer* with correlations being made with testicular development and male colouration at key times of the year.

3.2 Materials and Methods.

3.2.1 Sites.

Two sites were selected for monthly sampling over a twelve month period (February, 1985 to January, 1986), one at Deep Creek at Narrabeen (hereafter referred to as Deep Creek) and the other on the Lane Cove River about five kilometres upstream from the junction with Sydney Harbour. Both sites are in the metropolitan area of Sydney, N.S.W., but are in different catchment areas (Fig. 3.1). It was of interest to compare gonad development of *P. signifer* in a site that was heavily urbanised (Lane Cove River) with a less urbanised site (Deep Creek). According to Recher, Hutchings and Rosen (1993), with the exception of sub-fossils in Aboriginal middens, there are no historical data that provide an accurate review of changes on the distribution and abundance of aquatic organisms within the Hawkesbury-Nepean catchment.

Deep Creek.

Deep Creek, Narrabeen is situated at a latitude of 33° 42'S, 151° 15'E. It lies partly within the Garigal National Park. The catchment of the lagoon covers 55 square kilometres and the catchment area of Deep Creek is 15.8 square kilometres (Morris, 1993). Deep Creek is directly over Hawkesbury sandstone bedrock.

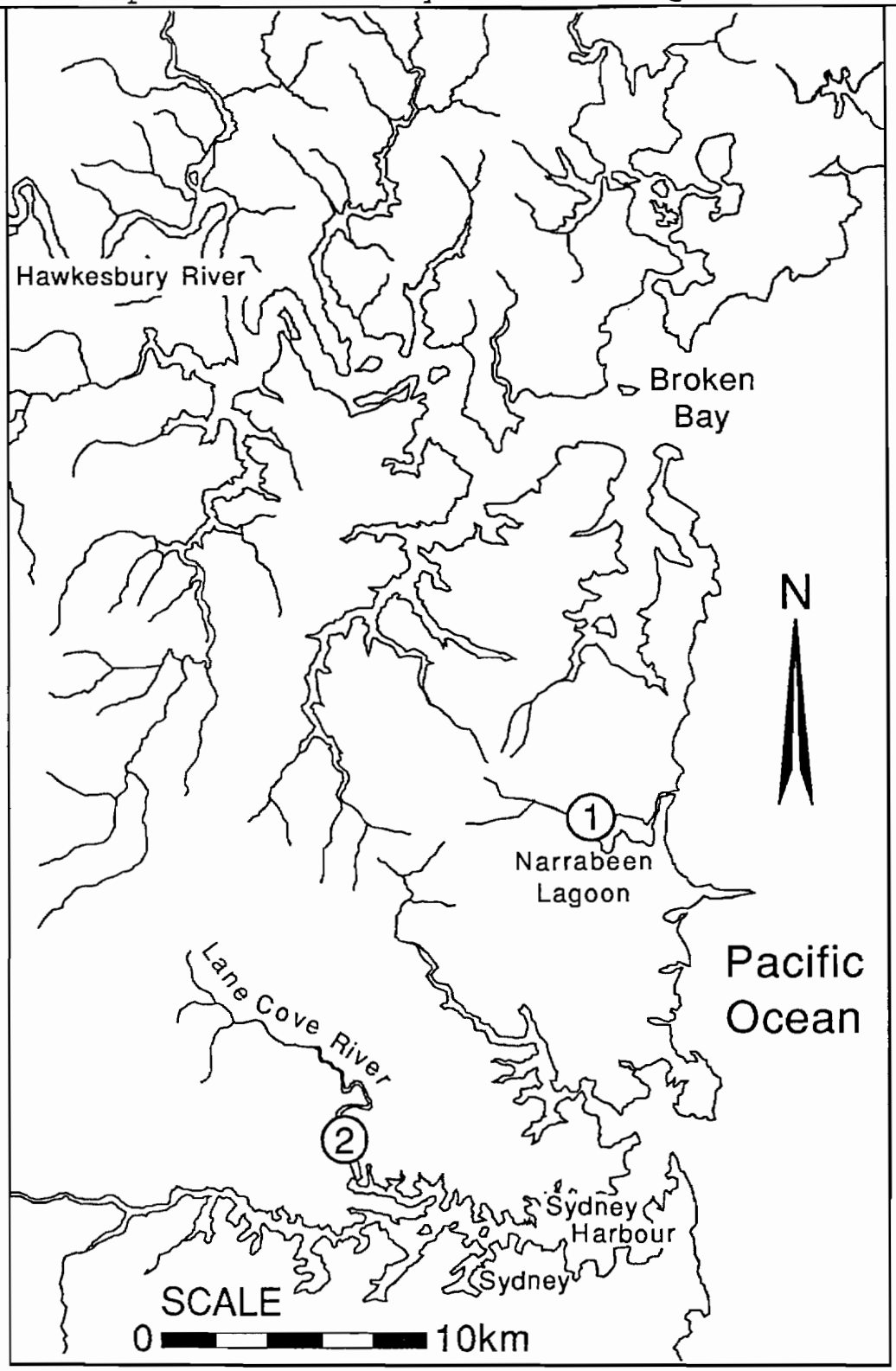


Figure 3.1
Location of sampling sites (Deep Creek, Narrabeen (1) and Lane Cove River (2)) used for the study of *P. signifer*.

The vegetation around the creek is composed of a mosaic of heath, scrub, woodland and open forest communities. The vegetation on the ridges was largely unaffected by exotic vegetation and human activity was limited, but vegetation along Deep Creek itself was somewhat disturbed (Benson, 1979). The entire catchment area was described as being relatively undisturbed overall (Benson, 1979). There is not much building development or industrial or domestic runoff along the catchment of the creek, though the Kimbriki waste depot is located at Terrey Hills within the catchment. The creek is used for passive recreation. Deep Creek catchment has only fairly recently (1960s) been opened for development (J. Davies, Conservation Dept., Warringah Shire Council, pers. comm.). At the time of this study the site was considerably less urbanised than the one at Lane Cove. The site at Deep Creek had a shallow seagrass bed, *Zostera capricorni*, at the edge of the Creek. The creek edges were shallow (only a few centimetres deep) and there was a drop to approximately 60 cm depth at approximately half a metre out from the bank's edge at mid-tide. Many snags in the form of rotting vegetation (branches and leaves) were observed along the creek. The substrate was composed of a fine silt with the odd patch of sand. *Casuarina* sp. lined the banks. There were no mangroves.

P. signifer and *G. holbrooki* were sampled from Deep Creek some 15 years ago for an investigation into prey size selection (Booth, 1980) but fish populations have not been surveyed systematically in recent years at Deep Creek.

Lane Cove River.

The sampling site was situated at a latitude of 33° 49'S 151° 08'E. Lane Cove River is part of the drowned river valley of Sydney Harbour. It cuts through Hawkesbury sandstone. The bottom is silty to peaty quartz sand, silt and clay with common shell layers. Including the tributaries, Lane Cove River drains a catchment of 93 square kilometres in the north western section of the Sydney Metropolitan Area (SPCC, 1980). This SPCC report found that the estuarine part of the River is of good quality during dry weather but during wet weather the estuary became polluted by stormwater carrying oxygen-demanding substances (e.g. sewage) and suspended matter. The report was a water quality study and no fish were sampled.

A list of fish compiled by the NSW State Fisheries and the Australian Museum,

for the Lane Cove National Park appears to be somewhat dated and possibly incomplete (Brown and Mitchell, 1983). There was no mention of *P. signifer* although *P. signifer* has been recorded as being caught in the Lane Cove River in the Australian Museum records in 1974 by Ivantsoff (J. Paxton, Australian Museum, pers. comm.).

A major sewer main, the Northern Sydney Ocean Outfall Sewer, has a stormwater overflow which discharges into Lane Cove River about one kilometre upstream of the Figtree Bridge in heavy rain. The overflow lies within the sampled area. The Lane Cove River catchment is heavily urbanised and has been for over fifty years. The site used for sampling was heavily vegetated with mangroves. The substrate was slightly rocky with a fine muddy bottom.

3.2.2. Fish collection.

The site at Lane Cove was in a mangrove peninsula in the Lane Cove River. The fish were generally caught at the edge of the mud bank or among mangrove pneumatophores. *P. signifer* was generally obtained from a 30-90 cm water depth. The hand net used did not permit sampling at greater than 1.5 metres.

No mangroves were present at Deep Creek. The seagrass *Zostera capricorni*, formed the habitat in which *P. signifer* was most commonly caught. Large numbers of *G. holbrooki* were caught in the net with *P. signifer*. *P. signifer* were obtained in less than 90 cm water, except in June when they were obtained from about 150 cm water. Sampling was undertaken at about mid-tide.

At an initial collection in January, approximately fifty fish from each site were retained and placed in 50 litre aquaria and gradually acclimated to freshwater over a period of two weeks. Methylene blue was added to the water to prevent fungal diseases. The captive population was maintained in Sydney and therefore experienced the same seasonal variation as the field specimens as they were at the same latitude. Every third month fish were randomly selected from tank populations for light microscopic examination of the gonads.

From February 1985 to January 1986 with the exception of December 1985, monthly sampling of both sites was undertaken. A two poled hand net, (115 by 115 cm, with a mesh size of 4 mm) was used, and a sustained sampling effort was made

for forty minutes or until 100 fish were caught. During some months no fish were caught using this method. The minimum size of *P. signifer* caught was 14 mm total length (TL) (mouth to the tip of the tail). Individuals over 30 mm TL were considered to be adults, at which size it is possible to recognise both males and females in the field. Fifty randomly selected fish were measured. Both TL and standard length (SL) (mouth to the caudal peduncle) measurements were obtained with a ruler. When available, each month five to ten male and female fish gonads from each site were fixed for light microscopy examination. Other species of fish caught in the net whilst in pursuit of *P. signifer* were identified and released.

3.2.3. Gonad examination - light microscopy.

The *P. signifer* for examination were fixed in Bouin's fixative for several days, and then washed in water. The fixative was removed by a series of alcohol treatments. The head and tail of the fish was then cut off. Decalcification in 5% nitric acid followed for 3 hours. Following a change of the acid, they were left to stand for a further 3 hours. To test for calcium 1ml of 5% sodium oxalate was added to 5ml of solution containing the tissue and the mixture was allowed to stand for five minutes. A precipitation indicated that decalcification was not complete and the process continued until a clean solution was obtained. The gonad was then removed by placing the fixed fish on its side and cutting away the flesh of the skin in a semicircle from the anus to just below the operculum.

Female fish.

During the summer months the ovary could be clearly observed as it filled the entire abdominal region. With the approach of winter it only filled a small portion of the abdominal cavity and was dissected out along with other tissue for fixation. The ovary (or the ovary and surrounding tissue) were fixed in Bouin's solution for several hours or days depending on the size of tissue, after which it was stored in 70% alcohol before being embedded in paraffin (Appendix IA). The fixed tissues were embedded in wax, sectioned at 7μm and stained with Harris' haematoxylin and eosin as described in Appendix IB following the procedure of Culling, Allison and Barr (1985).

Serial sections were made of the ovary. The size and composition of the oocyte population was quantified in each ovary. A classification of oocytes similar to that of Aldenhoven (1984) was developed as in Table 3.1.

Table 3.1. Classification of oocytes in *P. signifer* ovaries.

Class	Diameter (mm)	Recognition features
1	0.03-0.08	Cytoplasm strongly basophilic. (Stains strongly with basic dyes).
2	0.08-0.13	Cytoplasm less basophilic.
3	0.13-0.26	Appearance of lipid in cytoplasm.
4	0.26-0.52	Well developed zona radiata. Abundant yolk globules & vesicles.
5	0.52-0.92	Granular, yolky eggs, usually difficult to section. Stain deep purple in the central region. Well developed zona radiata, granulosa and theca cells.

Oocytes were counted and measured in 94 ovaries for the total study. In assessing oogenesis the presence or absence of each egg class was scored. The predominant egg class was also noted. The overall state of development was assessed according to the above classes. Fecundity was estimated by counting the number of oocytes in the largest size class (class 5 Table 3.1) of ripe ovaries. The number of large class 5 oocytes was quantitated from the serial ovarian sections using a modified method of Zuckerman (1951) as describe for mammalian reproduction. Class 5 oocytes were counted in every fifteenth section using the oocyte nucleus as a marker. It was determined that nuclei of class 5 oocytes were approximately 100μm in diameter and as the sections were 7μm thick it was unlikely that the same oocyte would be counted twice. Analysis of variance (ANOVA) was used to determine whether there were any differences in fecundity throughout the sampling period at the two sites.

Male fish.

It was difficult to dissect out only the testis in the nonbreeding season, therefore testis weights were not always obtained. The entire contents of the body cavity were dissected out in the majority of male fish. Fixation was performed in the same manner as for the ovary of female fish. Unlike the female, the male's gonad was not

always serially sectioned. In such cases sections were taken at the widest cross section to determine whether spermatogenesis was occurring. Testes were dissected out of 25 fish from the Lane Cove site and 24 from the Deep Creek site. The stages of testicular activity in male *P. signifer* were classified after the scheme of Harris (1986) as follows :-

1) Undeveloped - Small in size. Ducts open but very few spermatozoa present. They have a lobular structure but no lumens.

2) Ripening - Sperm duct has some spermatozoa.

3) Spawning - Large, occupying 1/3 - 1/2 the body cavity. Lumens very clear. Sperm duct full of spermatozoa.

4) Spent - Very small in size. No spermatozoa present. Cells have a homogeneous appearance.

Fig. 3.2 shows a typical section of an undeveloped testis, a developing testis and one that is in spawning condition.

Spermatogenesis was assessed by recording the abundance of later stages of spermatogenesis.

3.2.4 Water parameters.

The temperature was recorded monthly from each site and in the laboratory aquaria using a thermometer. Salinity measurements were made monthly at each site, by flame photometry, against a seawater standard.

3.2.5 Rainfall.

The Lane Cove site could be considered to have a similar amount of rainfall to that collected at the Sydney Regional Office of the Bureau of Meteorology and that of Narrabeen could be considered to have similar rainfall to that collected at the Newport collecting station by the Bureau of Meteorology.

Monthly rainfall data from February 1985 to January 1986 at these two collecting stations were compared with average monthly rainfall data. For the Sydney data the average monthly rainfall was calculated from data collected between 1858-1993 at the Sydney Regional Office of the Bureau of Meteorology.

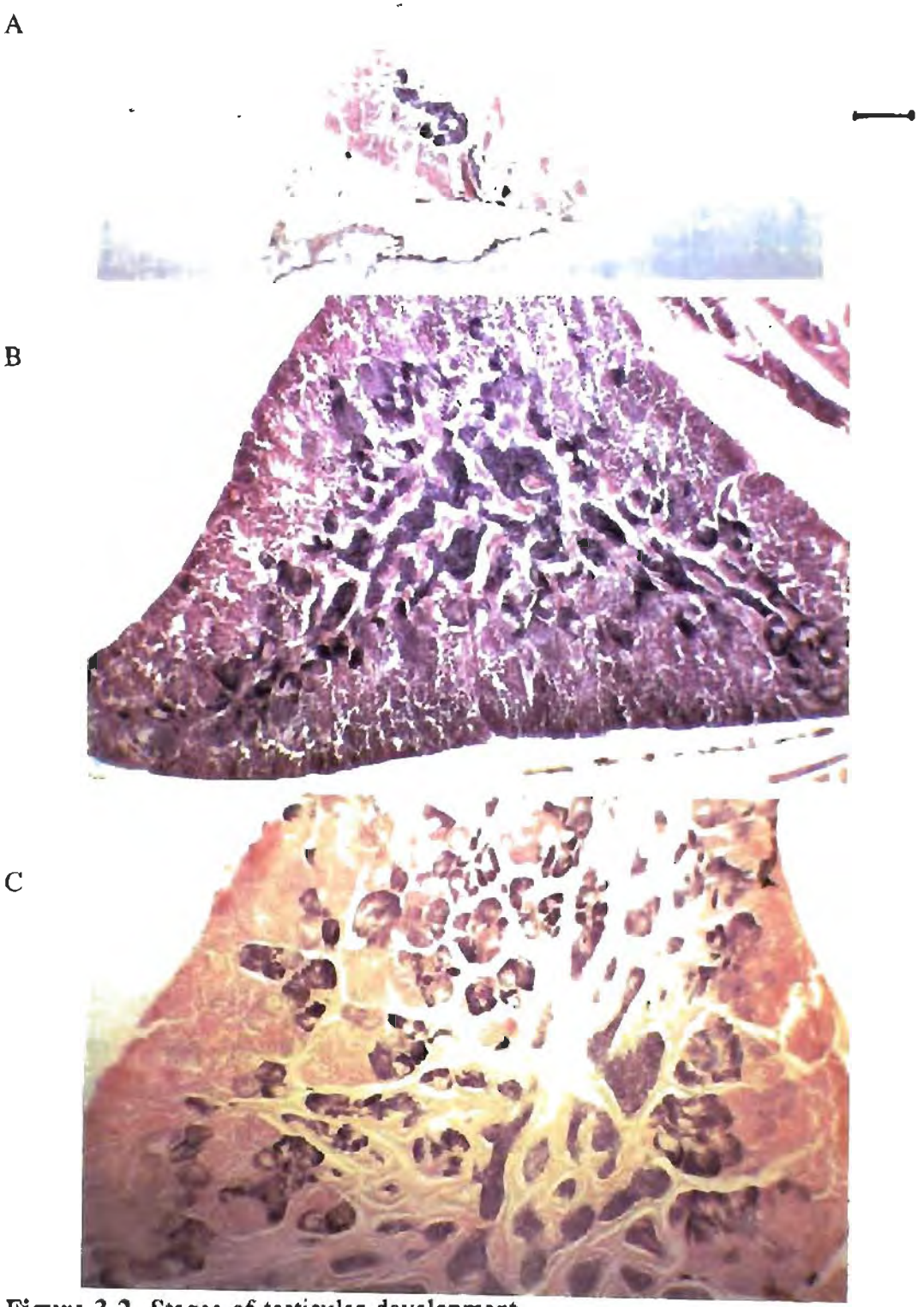


Figure 3.2. Stages of testicular development.
(A) Undeveloped testes with little spermatogenic development from an immature fish collected from Lane Cove in June, 1985.
(B) Developing testes from Lane Cove in February, 1985.
(C) Testes in spawning condition from a mature male showing the various stages of spermatogenesis collected from Lane Cove in October, 1985. All sections are the same magnification (scalebar equals 0.1 mm).

For the Newport data the average monthly rainfall was calculated from data collected between 1931-1993 at the Newport Collecting Station (Newport Bowling Club) of the Bureau of Meteorology.

3.3 Results.

3.3.1 Abundance.

The total number of fish caught by a uniform sampling effort each month, at each site, is shown in Fig. 3.3. The numbers caught were variable. None were caught in May and November at either site, or in July and August at Deep Creek.

Table 3.2 shows the numbers of fish identifiable as male or female caught throughout the year. During the period May to July it was not possible to reliably sex individuals.

3.3.2 Fish collection.

In addition to *P. signifer* a number of other species of fish were captured inadvertently. They were identified down to at least family, or further if possible, counted and released (Table 3.3)

3.3.3 Standard length.

Standard lengths of the captive fish and the wild caught stocks from February, 1985 to January, 1986 are recorded in Fig. 3.3.

Standard lengths remained relatively constant at Lane Cove River from February to October, with the final sampling in January yielding fish with a slightly greater standard length than all previous samples although this difference was not significant (one-way ANOVA). Linear regression analysis yielded no trend in standard length through the year at Lane Cove.

At Deep Creek, the mean standard length of fish caught increased steadily from March to October, with a significant decrease between October and January ($P<0.001$ one-way ANOVA with Tukeys post test). Linear regression analysis showed that the standard length increased in a linear fashion at a rate of 0.986 mm/month throughout this period ($r^2 = 0.9799$)

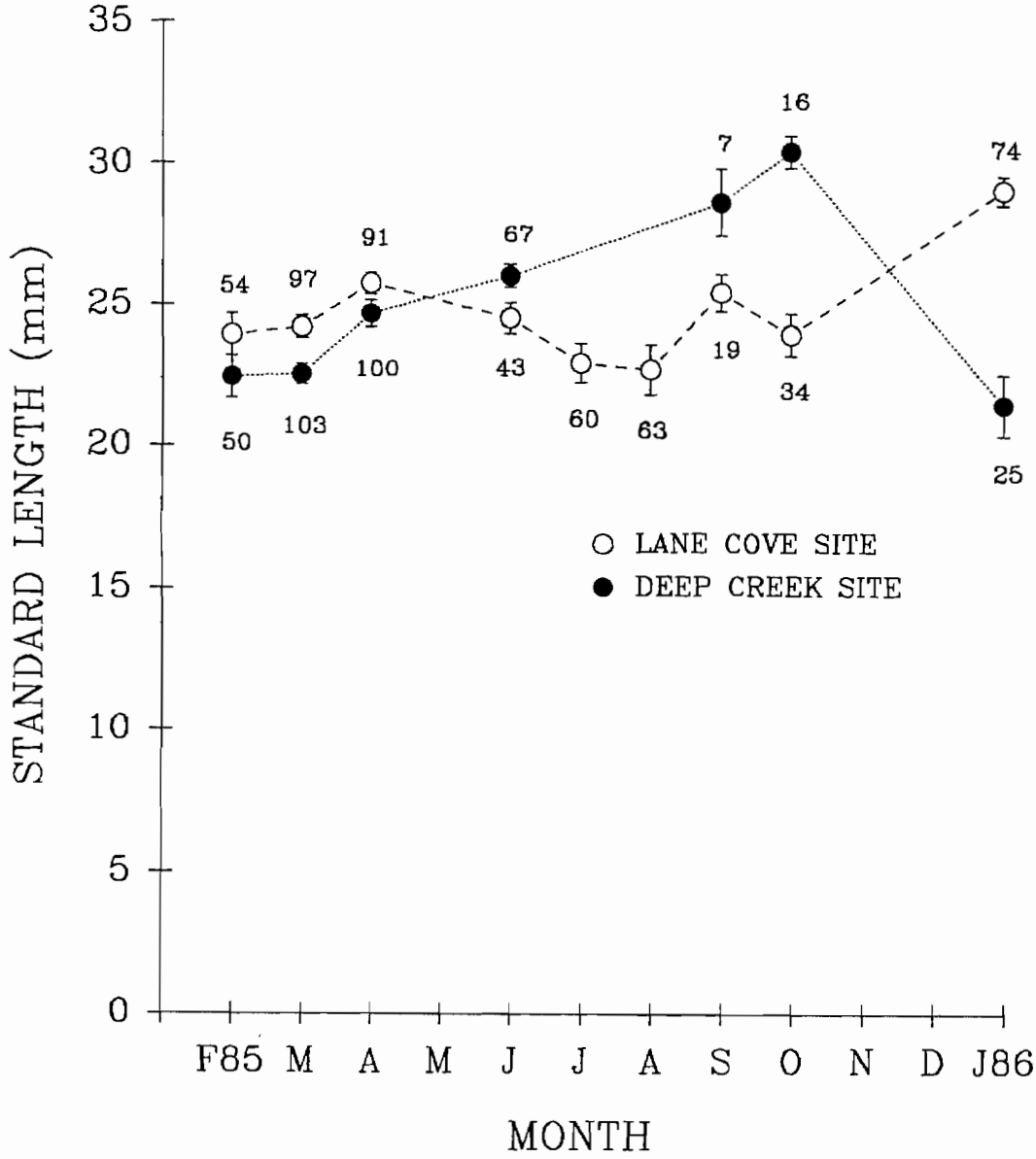


Figure 3.3. Standard lengths in mm (mean ± S.E.) throughout the year in *Pseudomugil signifer* collected at the two study sites at Deep Creek and the Lane Cove River. The numbers of fish caught at each site are indicated. No fish were caught in May and November at either site, or in July and August at Deep Creek. No sampling was carried out in December.

Table 3.2. Numbers of sexed *Pseudomugil signifer* caught. It should be noted that the sexes were not identifiable in the nonbreeding period. Not all the fish caught are represented in the table because their sex could not be determined at catch. Fish were caught in June at both sites and in July at Lane Cove but were not sexed unless gonad studies were performed. No *P. signifer* were caught in May and November at either site and in July and August at Deep Creek.

	LANE COVE		DEEP CREEK	
Month	Male	Female	Male	Female
Feb 85	10	22	3	6
March 85	10	10	12	4
April 85	15	16	9	5
May 85	0	0	0	0
June 85	not sexed	not sexed	not sexed	not sexed
July 85	not sexed	not sexed	0	0
August 85	18	15	0	0
Sept 85	10	9	4	3
Oct 85	8	17	5	11
Nov 85	0	0	0	0
Dec 85	no sample	no sample	no sample	no sample
Jan 86	47	25	4	5

Table 3.3 Numbers of other fish species caught at Lane Cove and Deep Creek whilst in pursuit of *P. signifer*. *S. hamiltoni- Spheroides hamiltoni* (Gray and Richardson)

	LANE COVE		DEEP CREEK	
Month	Number	Species	Number	Species
Feb 85	4	Gobiidae	0	
March	0		0	
April	0		0	
May	0		3	Gobiidae
June	0		0	
July	0		2	Gobiidae
August	0		0	
Sept	0		1 4	*G. holbrooki* Eleotridae
Oct	2 3 3	*S. hamiltoni* Gobiidae *Mugil* sp.	80 32	*G. holbrooki* Gobiidae
Nov	0		70	*G. holbrooki*
Jan 86	7	Gobiidae	>100 5	*G. holbrooki* Gobies

The lack of samples between October and January at Deep Creek make it difficult to know whether the standard lengths recorded at Deep Creek in January are an isolated result or whether a decline occurred in this period. However the standard length for January 1986 was not significantly different from those in February or March 1985.

At Deep Creek the maximum standard length coincides with the maximum ovarian weights.

3.3.4 Gonads.

Ovarian weights and the colouration of the male fish are recorded in Fig. 3.4. The testes were extremely small in the winter months. *P. signifer* were found to have a single ovary on dissection. Table 3.4 shows the major oocyte classes found in the captive and natural populations of *P. signifer* from February, 1985 to January, 1986.

More complete data were available on ovarian weights for the Lane Cove River site. Ovarian weight increased from minimum weights recorded from April to July, reaching a peak in October. Less data were available for Deep Creek, due to the difficulty of catching specimens during this time. The pattern at Deep Creek seemed similar to that at Lane Cove, despite the discontinuous data. In both cases an analysis by ANOVA revealed that ovarian weights fell into two groups according to the time of year ($P<0.001$). The period from March to August represented low ovarian weights and the period from September to January represented high ovarian weights (ANOVA with Tukeys post-test).

Breeding colour in the male could be identified as early as July in males at Lane Cove River, and was present thereafter to October. It was noted that colouration and ovarian weight increased prior to the appearance of the largest (class 5) oocytes, which appeared from August onwards (Table 3.4). When ovarian weights were in decline following the peak in October, class 5 oocytes continued to predominate until January.

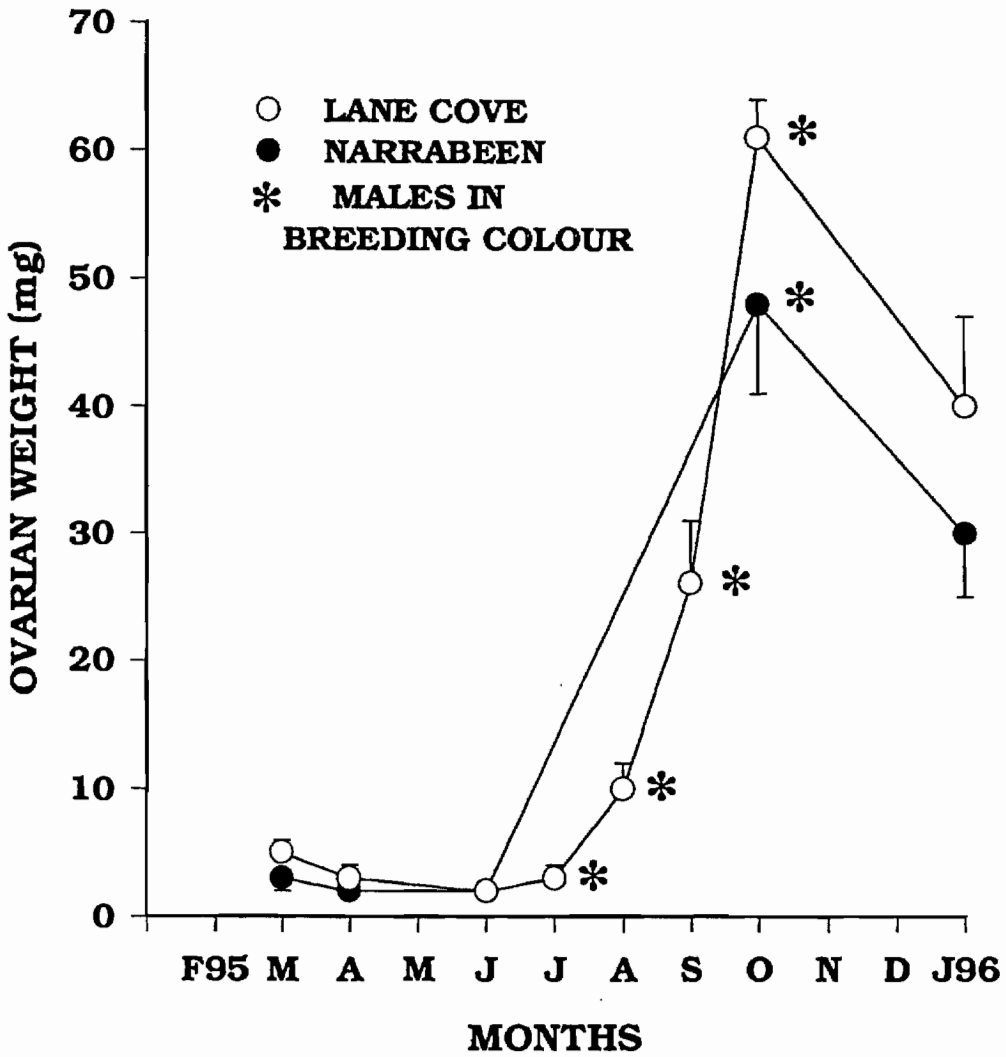


Figure 3.4. Weight of fixed ovaries from *Pseudomugil signifer* captured at the two study sites at the Lane Cove River and Deep Creek. Mean and standard errors are shown. Months in which captured male fish were in breeding colour are shown by an asterisk.

Table 3.4. Ovarian development, from February 1985 to January 1986. The most advanced oocyte class found in each fish is recorded, following the classification in Table 3.1. For example, in February, 1985, at Lane Cove, 7 wild-caught fish were examined, 6 had Class 5 oocytes and 1 had Class 2 as the most advanced oocytes found.

MONTH	LANE COVE		DEEP CREEK	
	Wild	Tank	Wild	Tank
Feb 85	6/7 class 5 1/7 class 2	-	6/6 class 2	-
Mar 85	1/5 class 2 2/5 class 3 2/5 class 4	3/3 class 1	2/5 class 2 3/5 class 3	3/4 class 3 1/4 class 5
Apr 85	5/6 class 3 1/6 class 4	-	5/5 class 3	-
May 85	-	2/2 class 1	-	1/1 class 3
Jun 85	2/5 class 2 3/5 class 3	3/4 class 2 1/4 class 3	5/5 class 3	1/2 class 3 1/2 class 4
Jul 85	3/6 class 3 3/6 class 4	-	-	-
Aug 85	5/5 class 5	-	-	-
Sep 85	5/5 class 5	1/3 class 4 2/3 class 5	1/1 class 5	1/1 class 5
Oct 85	2/2 class 5	-	5/5 class 5	-
Jan 86	2/2 class 5	-	2/2 class 5	-

From April to July the ovary contained mostly small oocytes (classes 1,2 and 3) with only a few class 4 and no class 5 oocytes indicating a decline in reproductive activity at both sites. The three photomicrographs in Fig. 3.5 show that the size of the ovary increased from the non-breeding to breeding season. During the breeding season the ovary enlarged from its elongate appearance to fill the entire body cavity. Smaller oocytes were seen in the Lane Cove captive group than in their wild counterparts.

From August to February class 5 oocytes (the fish were in spawning condition) were found in fish from the Lane Cove River site. From July the large males developed coloured dorsal and anal fins. However, it was not until August that large amounts of spermatogenic activity were observed in their testes.

Table 3.5.
FECUNDITY ± Standard error

Month	Lane Cove	Deep Creek
Feb,85	12.3 ± 1.3 n=6	0 n=6
Mar,85	0 n=5	0 n=4
April,85	0 n= 6	0 n=5
June,85	0 n=5	0 n=2
July,85	0 n= 6	no fish caught
August,85	32.8 ± 3.1 n=5	no fish caught
September,85	38 ± 3.3 n=5	61 n=1
October,85	43 ± 5.0 n=2	53 ± 7.4 n=5
January,86	17.5 ± 10.5 n=2	18 ± 0.6 n=5

The results from Deep Creek were a little more difficult to interpret as no fish were caught in August and fish of a smaller standard length in comparison to the Lane Cove River site were caught in February and March. However, fish from Deep Creek were found to have class 5 oocytes in September, October and January (when males had bright colours and active testes) but not in February or March as would be expected from juvenile fish. The results of the measurements of fecundity are presented in Table 3.5. Fecundity was significantly higher in the period from August to October than those from January to February. In turn January and February measurements of fecundity were higher than those from March to July.

At Deep Creek no *P. signifer* were caught when the water temperature was less than 14°C (May to August) except in June, when they were caught with difficulty in deeper water. At this time the males displayed no breeding colouration, and the females had limited activity in their ovaries (mostly up to class 3 oocytes).

P. signifer were found to have only one testis, which was elongate in appearance. In fish in non-breeding condition (April-July), the testes assumed a very fine threadlike appearance or regressed to the point where they could not be seen at all on dissection. During the breeding season (August - February), they became much heavier in appearance and filled 1/3 - 1/2 of the body cavity.

When fully active the testis is large and lobular in structure with spermatozoa centrally located within the sperm duct and developing cells radiating out to the periphery. In cross section the testis was roughly triangular in appearance. Four stages of spermatogenesis were recognised. These were spermatogonia (mean nuclear diameter 3.8 μm), spermatocytes (mean nuclear diameter 2.6 μm), spermatids (mean nuclear diameter 2.0 μm) and spermatozoa (mean nuclear diameter 1.3 μm).

Tables 3.6 and 3.7 give an indication of the stages of testicular development of *P. signifer* from the two sites.

3.3.5 Water quality parameters.

Salinity, temperature and pH were routinely measured at each collection site and in the aquaria.

Fig. 3.6 depicts monthly salinity readings of the water at the two sites over the one year cycle. It should be recalled that the captive fish were acclimated to fresh water at the start of the experiment.

After commencing in February, 1985 at similar salinity levels, approaching that of seawater, both sites experienced declines in salinity coincident with unusually heavy rainfall in April, 1986 (Fig. 3.7). Thereafter, salinity levels at Deep Creek were considerably lower than at Lane Cove River. Salinity at Deep Creek did not exceed 15 g/l, while at Lane Cove River, salinity did not fall below 20 g/l for the remaining duration of the study. Transient increases in salinity occurred at both sites, commencing in August, which had unusually low rainfall. Salinities had declined again by the close of the experiment, with Deep Creek being fresh water.

Fig. 3.8A shows the daylight pattern for Sydney for the period February, 1985 to January, 1986. Winter solstice was at 21st June, and the summer solstice was at 22nd December. Fig. 3.8B shows monthly readings of temperature at the field sites and in the laboratory aquaria. All follow a similar pattern, with nadir of temperature occurring in June or July and maximum temperatures in January, February or March.

Table 3.6. Development of testes from February 1985-January 1986 in *P. signifer* collected from Deep Creek.

Month	Development	Ducts empty or full	Lobular
February 85	Different stages but not many spermatozoa	Full	No
March 85	All stages	Full	Yes
June 85	All stages, gonad small	Some	No
October 85	All stages very developed	Full	Yes
January 86	All stages very developed	Full	Yes

Table 3.7. Development of testes from February 1985-January 1986 in *P. signifer* collected from Lane Cove River.

Month	Development	Ducts empty or full	Lobular
February 1985	All stages very developed.	Full	Yes
March 1985	Stringy appearance, not developed.	Empty	No
April 1985	Atrophied.	Empty	No
June 1985	Different stages, different cells at exterior of gonad.	Some	No
July 1985	Different stages, but testes were spent.	Empty (2) Few sperm (2)	No
August 1985	All stages very developed but not quite as developed as February.	Full	Yes
September 1985	All stages well developed.	Full	Yes
October 1985	All stages well developed.	Full	Yes
January 1986	All stages well developed.	Full	Yes

The range of temperatures observed in the laboratory (14.5-24°C) was less than that observed in the field (10.5-27°C). The lowest temperatures observed were at Deep Creek in June and July (10.5°C). Temperatures at Lane Cove did not fall below 13°C.

Months when no fish were caught from the wild (4 occasions) are marked on the graph (Fig. 3.8B). Non capture did not appear to be related to temperature.

The pH of the water at both sites, and in the tanks, varied very little and remained in the range 5-6 during the period of study.

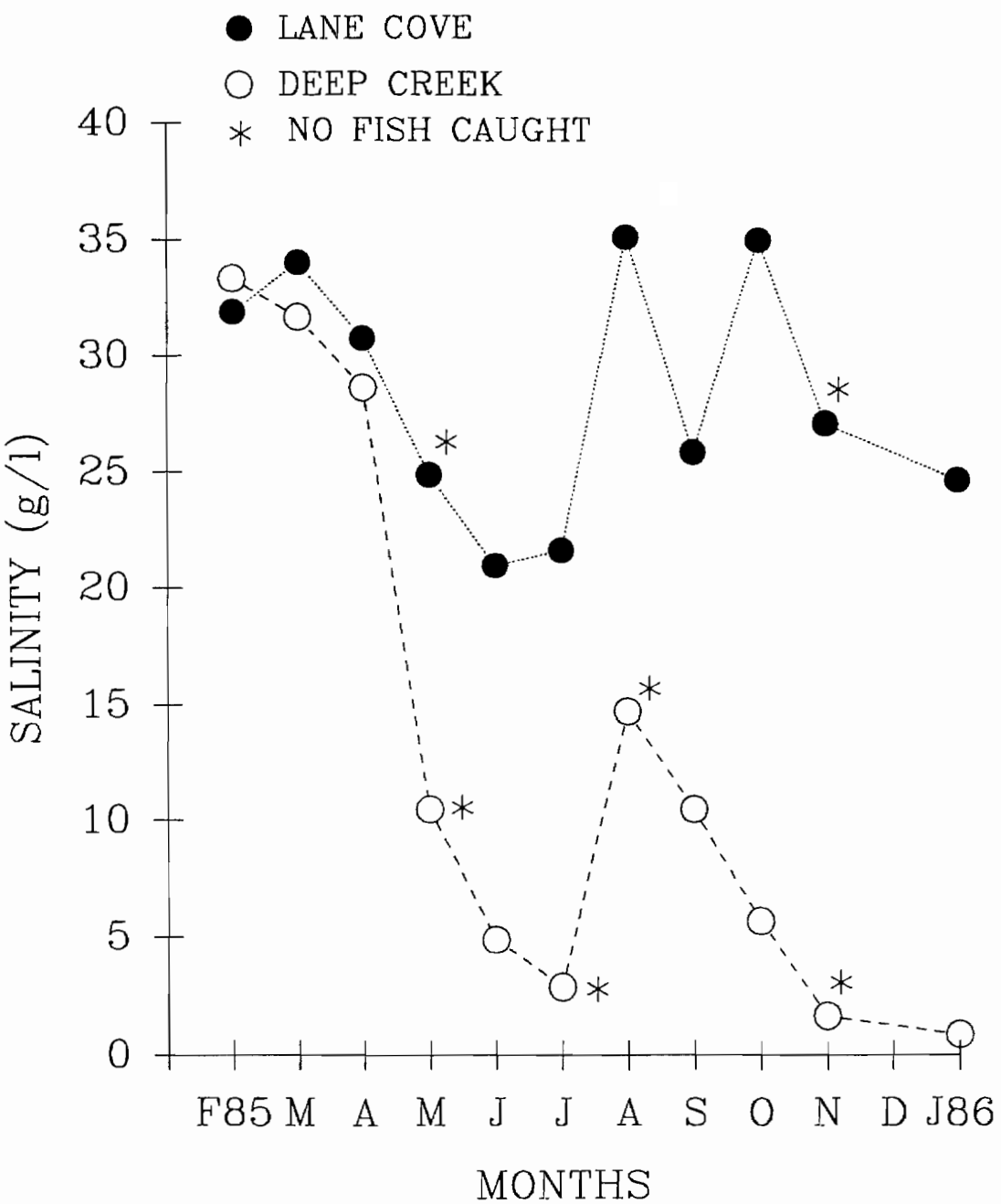


Figure 3.6. Salinity of water, estimated by flame photometry, and expressed as g/l, at the sampling sites at Lane Cove and Deep Creek (captive colonies were maintained in freshwater). Occasions on which no fish were caught on sampling are shown by an asterisk.

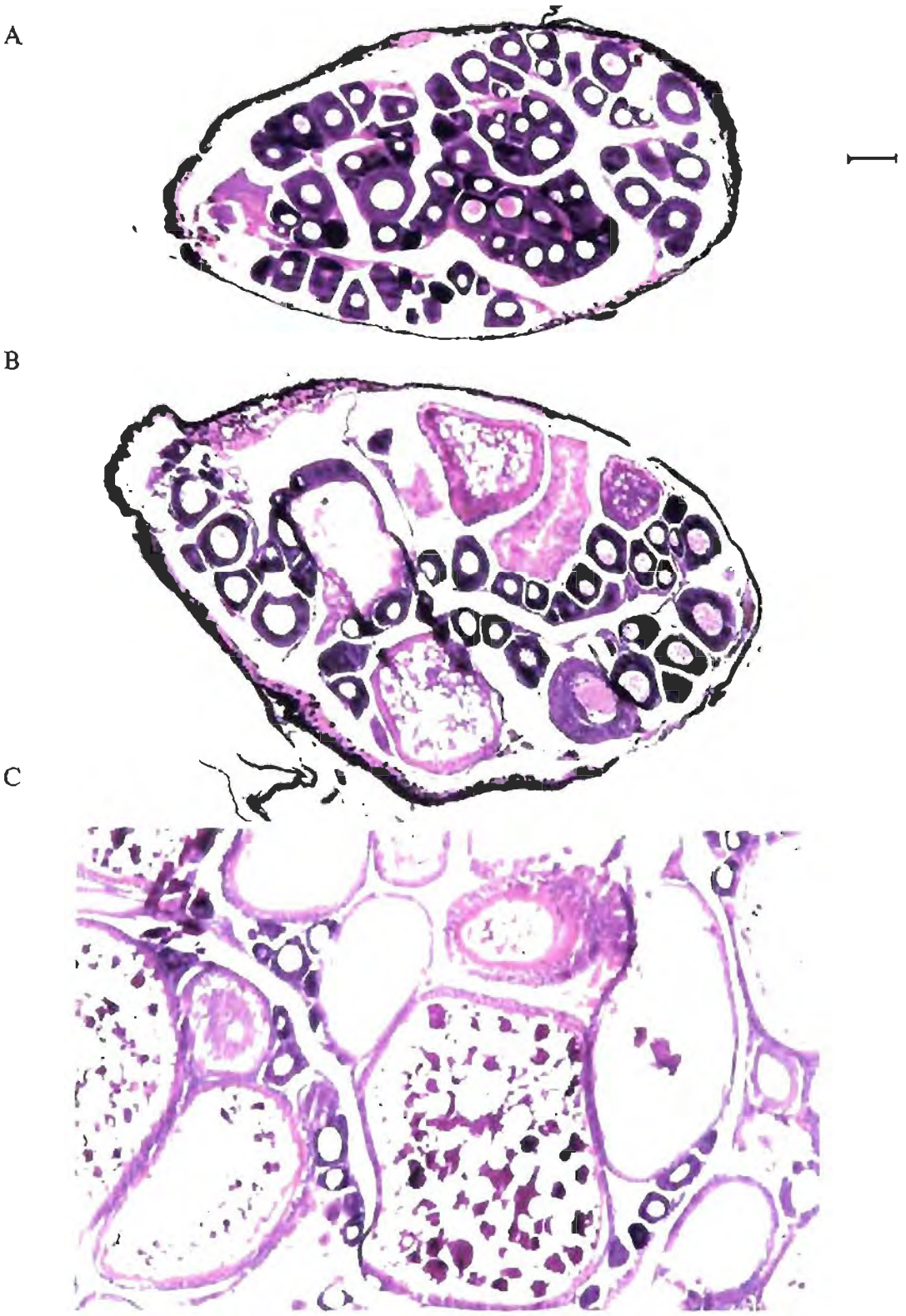


Figure 3.5. Ovarian photomicrographs from *Pseudomugil signifer* at various stages of their breeding cycle. (A) May 1985 - Tank specimen, Lane Cove River colony. Small oocytes only were observed. (B) July 1985 - Wild specimen, Lane Cove River. Up to class 4 oocytes but no class 5 (non breeding). (C) October 1985 - Wild specimen, Deep Creek. Class 5 oocytes present (full breeding condition).
All sections are at the same magnification (scale bar equals 0.1 mm).

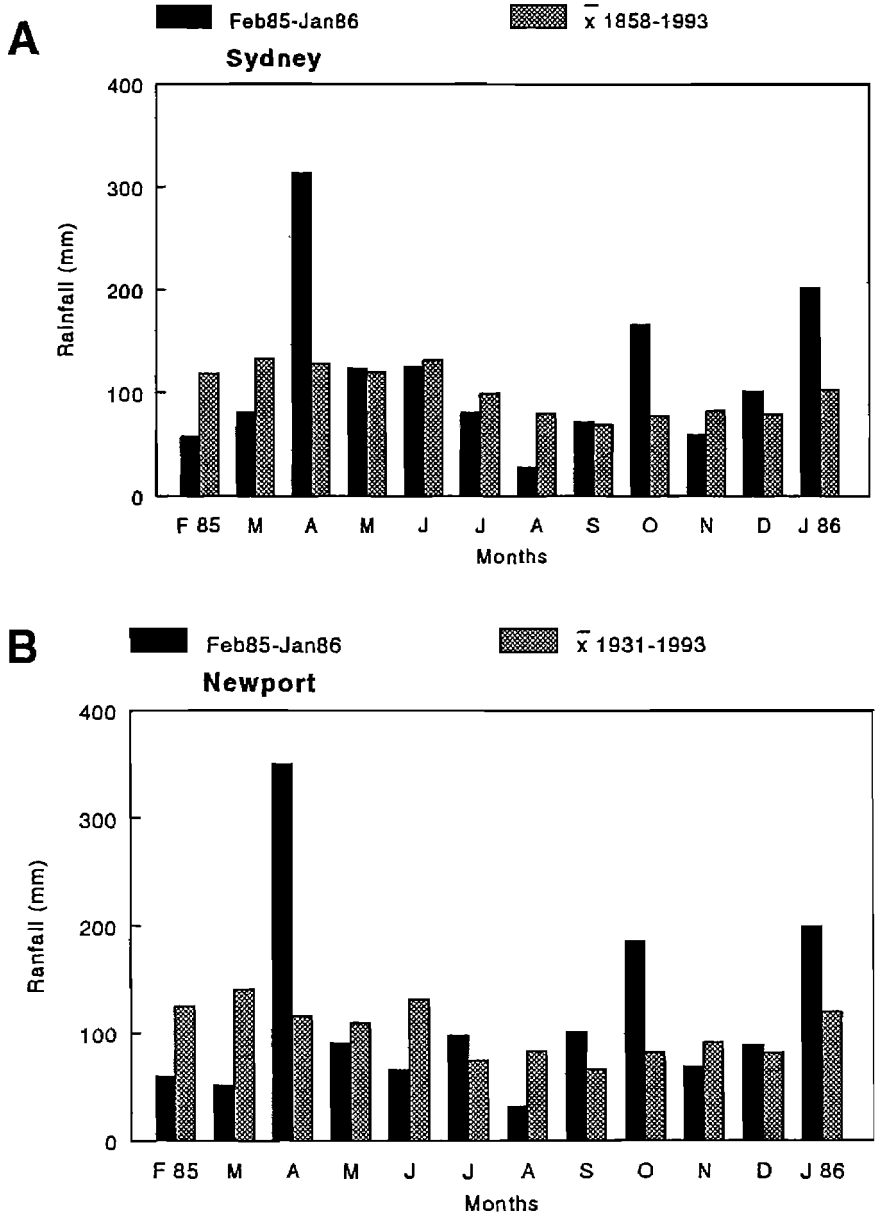


Figure 3.7. Average monthly rainfall at Sydney (A) and Newport (B) from February 1985 to January 1986 with a comparison of average monthly levels from data collected from 1858-1993 at Sydney and 1931-1993 at Newport. (Bureau of Meteorology, New South Wales Regional Office).

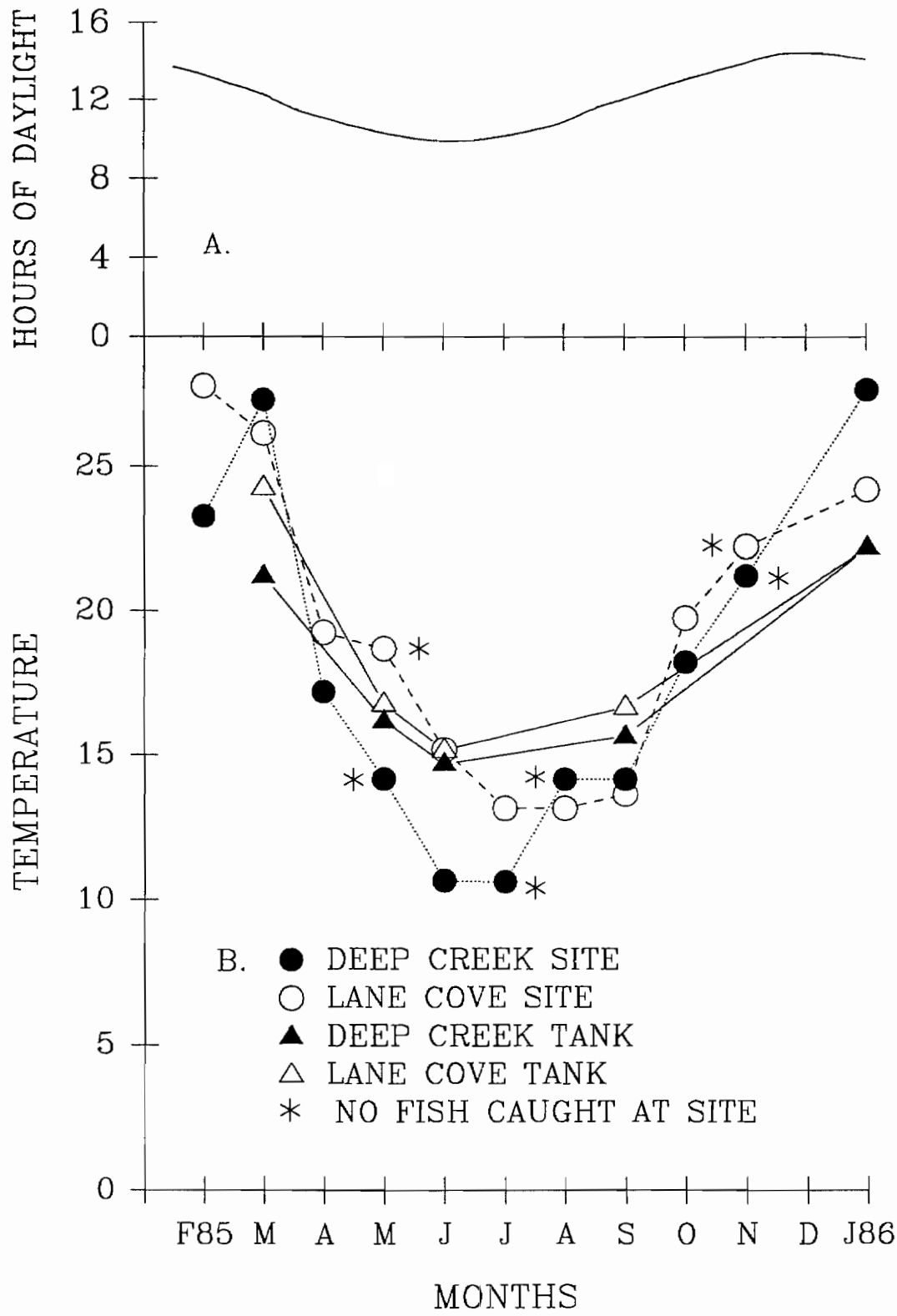


Figure 3.8 (A) Hours of daylight throughout the year at the latitude of Sydney, NSW. (Bureau of Meteorology New South Wales Regional Office). **(B)** Water temperature throughout the year at the sampling sites at Lane Cove River and Deep Creek, and in the captive colony aquaria. Occasions on which no fish were caught on sampling are shown by an asterisk.

3.3.6 Rainfall.

The pattern of rainfall was very similar at both collecting sites. The monthly rainfall at both collection points (Sydney and Newport) was higher than average for the months of April, September, October and December in 1985 and January, 1986. However, at Sydney the total rainfall for this period of 1414.2mm was only slightly above the mean of 1226.8 mm (for the period 1858-1993). The total rainfall at Newport of 1392.6mm was also only slightly higher than the mean of 1242.4mm for this station over the period 1931-1993 (Fig. 3.7).

3.4 Discussion.

A study carried out over two or more years would normally be more appropriate to look at seasonal effects on the reproduction of fish. However histological studies of the ovarian development of the freshwater drum *Aplodinotus grunniens* Rafinesque have recently been carried out over a thirteen month period including a period of no sampling between October and April due to ice formation (Palmer *et. al.* 1995). The resources available for the present study allowed only for one year of sampling to be performed.

3.4.1 Abundance and reproductive condition.

The results showed that patterns of abundance and reproductive condition in *P. signifer* from the two sites studied were related to seasonal environmental conditions. Seasonal changes in the abundance of *P. signifer* were noted at each site. Numbers caught for the same sampling effort were variable. This variability and the failure to catch any fish on a number of occasions, may reflect the discontinuous distribution of the fish in schools. Nothing is known of whether the species forms schools of mixed age structure or whether similar age groups school together. The methods used precluded capture of the smallest fish.

In addition, using ease of capture employing the described technique as a guide, it seems that *P. signifer* were more abundant at the Lane Cove River site. No fish were obtained at Deep Creek in May, July and August. These data suggest that the mangroves in the more open estuarine environment of Lane Cove River were the preferred habitat. Considerable effort was made to equalise sampling effort by using

the same operator, sampling for the same length of time and in the same place at each site. The two sites were sampled on the same day, to provide spatial replication. However all sampling methods have disadvantages and it is acknowledged that the sampling method used in this study probably did not allow the collection of large numbers of fish. It did allow sampling in the difficult area of the mangroves at the Lane Cove site.

3.4.2 Possible factors that might affect abundance.

P. signifer is known to be euryhaline and is found in a great variety of habitats (Allen and Cross, 1982). Therefore, the lower salinity as a single factor at the Deep Creek site would be unlikely to affect their abundance. Comparable results have been reported from another study; not many fish were found in creeks of a subtropical saltmarsh in the winter months (Morton *et al.*, 1988). The minimum winter surface temperature at Deep Creek was lower than that at Lane Cove River. It was not possible to sample in water deeper than 1.5 m with the available equipment. *P. signifer* would be older and larger by winter and may have been sensitive to the cool temperatures. Possibly the fish went to deeper, warmer water at those times when they could not easily be obtained by hand net in mid winter. Those individuals caught in June were obtained from water about 1.5 m deep, which is consistent with this suggestion. The number of fish caught appeared to be related to the temperature of the sampling site.

The pH in the field is lower than thought acceptable for fish culture (pH 6.5-9.0) (Cook, 1986). It is of interest that the literature does not provide very many data for water quality parameters for fish in Australia.

In addition, *G. holbrooki* were found only at the Deep Creek site. The presence of this species may have been detrimental to the success of *P. signifer*, as they appear to require similar resources, a point which is discussed further in Chapter 5. *G. holbrooki* are prolific live bearers and hence are considered by some authorities to present a threat to small native fishes (McDowall, 1980). In contrast, *P. signifer* are not very prolific and are egg layers (Howe, 1987).

3.4.3 Reproductive cycle.

Field information gathered on gonad development and secondary sexual characters along with data from the equivalent captive population, indicate that *P. signifer* has a well defined annual reproductive cycle. In mid-winter *P. signifer* did not show any indication of breeding in the wild. The gonads were small and inactive in both sexes and males lacked colour. These observations correlated directly with cessation of breeding under aquarium conditions. The breeding colour of the males appeared again in July at the Lane Cove River site before the advent of full spermatogenic activity in August. The first field appearance of full ovarian activity as judged by the by an increase in fecundity was in August. In September, all females from both sites had class 5 oocytes indicative of the breeding season. The highest fecundities were found in October at the same time at the largest increase in ovarian weight. No fish were caught in either November or December but by January there was a significant decrease in fecundity although class 5 oocytes were still present and *P. signifer* continued to breed in the laboratory. Male *P. signifer* continued to have brightly coloured fins throughout spring and summer which correlated well with ovarian activity. From March to July there was a decline in reproductive performance as evidenced by a decrease in ovarian weight and fecundity (lack of class 5 oocytes) in In addition the males showed no colouration during this period. The male colouration precedes spermatogenesis and may trigger the final stage of oogenesis in the female.

3.4.4 Information obtained from different sites and laboratory study.

Wild and captive populations were clearly different in their levels of reproductive activity throughout the year (Table 3.4). However, the difference was one of degree, both wild and captive groups showing a non-breeding state in mid-winter and increasing breeding activity towards summer.

3.4.5 Factors that might affect growth and reproduction of *P. signifer.*

The ovulation process in fish is thought to be regulated by an endogenous process in which a preovulatory elevation of blood gonadotrophin stimulates the production of ovarian steroids that cause oocyte maturation (Stacey, 1984). The latter suggests

that this is accomplished by two different methods:-

(1) Ovulation occurs as a result of completed ovarian development.

(2) Ovulation occurs in response to exogenous factors.

Light and temperature, acting via hypothalamic-pituitary-gonadal axis have been considered the main environmental factors regulating gametogenesis in seasonally breeding fish (Dodd and Sumpter, 1984). de Vlaming (1972) observed that the effects of photoperiod cannot readily be separated from those of temperature. *P. signifer* have been shown by the current study to be seasonally breeding fish which commence breeding as the temperature and daylength increase and decline in breeding activity as the daylength and temperature decline. It was observed that the water temperatures were low when gonadal activation commenced suggesting that photoperiod may be of more importance in triggering gonadal activity in *P. signifer.* For the site for which the most data is available (Lane Cove) the increase in ovarian weight does not lag behind the increase in temperature. It appears to be as near to simultaneous as can be deduced from monthly sampling. The completion of vitellogenesis in *P. signifer* could be responding to photoperiod as it lags by about a month whereas if it was responding to temperature the changes appear to be too rapid. The only way to separate the contributions of the two environmental factors would be through captive experiments, where photoperiod and temperature could be individually controlled.

Further, systematic studies on the conditions of captive maintenance would be worth pursuing to distinguish more definitively the factors which are important in the success of this species in various habitats. This type of study has been done with the complex reproductive strategies of the Australian Bass *Macquaria novemaculeata* in the Sydney Basin (Harris, 1986). Harris demonstrated that decreasing water temperature was a main environmental cue for the onset of spermatogenesis. In the case of *P. signifer* an increase in water temperature appears to be one of the major factors influencing spermatogenesis and oogenesis. Milton and Arthington (1984) also considered rising water temperature to be a stimulus to spawning in *Melanotaenia splendida fluviatilis.*

Biological factors, such as the presence of the exotic *G. holbrooki* are also likely to have an effect on reproduction and growth of *P. signifer* in the wild. The next

section of work presents the results from tank experiments which indicate clearly that *G. holbrooki* has an adverse impact on the growth and reproduction of *P. signifer*, at least in confined conditions.

Chapter 4.

Impact of *Gambusia holbrooki* on growth and reproduction of *Pseudomugil signifer* (experimental study).

4.1 Introduction.

It was found in the study of populations of *P. signifer* at Lane Cove River and Deep Creek, Narrabeen (Sydney) (Chapter 3), that seasonal environmental factors influence the normal breeding cycle of *P. signifer* (Howe and Howe, 1991). In that study it was also observed that where *G. holbrooki* was present (at one site, Deep Creek), *P. signifer* appeared to be less abundant at that site.

Gambusia holbrooki was introduced to Australia as a mosquito control agent, and has since become widely established. A number of authors (Allen, 1982,; Lloyd, 1982; Arthington *et al.*, 1983 and Merrick and Schmida, 1984) have speculated on the deleterious effects of *G. holbrooki* upon native fish species. In the study reported in this Chapter, the effect of the exotic species, *G. holbrooki*, on the reproduction and growth in the native species, *P. signifer*, has been examined under controlled captive conditions.

G. holbrooki has been cited several times as a pest in Australian waterways (Allen, 1982,; Lloyd, 1982; Arthington *et al.*, 1983 and Merrick and Schmida, 1984). In the current project, based on the study of seasonal reproductive activity in two populations of *P. signifer* (Chapter 3), it was postulated that the presence of *G. holbrooki* is detrimental to *P. signifer*, since they appear to require similar resources. The two species frequently share the same habitat along the east coast of Australia (Merrick and Schmida, 1984).

As pointed out in Chapter 3, *G. holbrooki* is a prolific live bearer and is considered by some (McDowall, 1980) to present a threat to small native fishes. *P. signifer*, on the other hand, lays small numbers of vulnerable eggs (Howe, 1987). The evolutionary histories of these two species are completely different. *G. holbrooki* is a New World cyprinodont of the family Poeciliidae, distinguished by an elongated gonopodium or intromittent anal fin in the male. Poeciliids evolved in Central America and the West Indies (Innes, 1966). *P. signifer*, on the other hand, belongs to a distinct Old World atherinoid family (Saeed *et al.*, 1989).

Pseudomugilids appear to have evolved in Australia, Papua New Guinea and some eastern islands of Indonesia.

A similar situation with respect to the biogeography of native fish species can be found in the western states of the U.S.A., as in Australia and New Zealand (Crowl, *et al.*, 1992). Douglas, *et al.*, (1994) proposed that the fish fauna of the American west was isolated and depauperate, having evolved in a time of disruptive geologic and climatic events (Minckley, *et al.*, 1986). This isolation led to the loss of defensive and competitive abilities (Moyle *et al.*, 1986, Douglas *et al.*, 1994). Similarly, the diversity of the freshwater fish fauna of Australia is poor, and has evolved in isolation (Merrick and Schmida, 1984), leading to a large proportion of vulnerable endemic species.

Because of the isolation of Australia's freshwater species, the introduction of the resilient, productive *G. holbrooki*, without any of its original competitors or predators, could have a great impact on our smaller native species, particularly endemic species occupying very restricted ranges. Lloyd (1990) suggested that long and short term coexistence experiments and behavioural studies would be of value in providing clear evidence of the impact of *Gambusia* on selected native fish.

Part of the major objective of this project, therefore, has been to identify factors affecting *P. signifer's* reproductive biology. This section of the study was designed to establish whether *P. signifer* is threatened by *G. holbrooki* and, by inference, whether other, more restricted pseudomugilid species such as *P. mellis* and *Scaturiginichthys vermeilipinnis* are similarly at risk. *S. vermeilipinnis* was first described in 1991 from Central Queensland, in the Lake Eyre drainage division (Ivantsoff, Unmack, Saeed and Crowley, 1991). *Gambusia holbrooki* was found with *S. vermeilipinnis* in three of five springs surveyed (Jackson, 1994). *G. holbrooki* were observed to nip the caudal fin of *S. vermeilipinnis* (Unmack and Brumley, 1991). *G. holbrooki* appeared to have a preference for deeper pools up to 8cm deep, and it was suggested that *S. vermeilipinnis* survived best in pools of 4cm depth where *G. holbrooki* were less prevalent. This spatial partitioning gives *S. vermeilipinnis* a refuge from *G. holbrooki*, but if such refuges were not available *S. vermeilipinnis* might rapidly become locally or generally extinct.

The interaction of *P. mellis* with *G. holbrooki* is not known but *G. holbrooki* are found in several localities with *P. mellis* (Wager and Jackson, 1993 and Jackson, 1994). Any data collected on *P. signifer*'s interaction with *G. holbrooki* could perhaps be extrapolated to *P. mellis*. *G. holbrooki* and *Xiphophorus maculatus* (platy; Poeciliidae) were absent when *P. signifer* comprised 73% of the fish caught from College's Crossing on the Brisbane River in 1977, but in 1981 both exotic fishes were present and *P. signifer* then comprised only 1.5% of the fish caught (McKay, 1984). It would appear from the above information that *G. holbrooki* threatens species from the family Pseudomugilidae, although systematic studies are lacking.

Two hypotheses have been proposed to explain how exotic species affect native western American fish (Douglas *et al.*, 1994). Because of the similarities in biogeography and human interventions these may be applicable to the Australian situation. The first hypothesis suggests that environmental degradation (generally caused by human activity), eliminates the native fish, allowing the introduced species to replace them (Douglas *et al.*, 1994). The second hypothesis suggests that direct displacement of the native by the introduced species occurs through impacts including such factors as predation by the exotic on the native species (Meffe, 1983), hybridisation (Echelle, 1991), competition for any of a variety of resources (Courtenay and Hensley, 1980), or a combination of effects.

G. holbrooki is an exotic species which has come into contact with *P. signifer* only because it has been introduced by humans. The introduction of this species has potential major implications for the conservation of a number of our smaller native species. *P. signifer* was selected as the model in these pest-species impact experiments for several reasons. They:-

- have an extensive range down the east coast of Australia (Merrick and Schmida, 1984);
- are abundant within their range;
- are relatively easy to catch;
- breed in freshwater in captivity (Howe, 1987; Chapter 3);
- have a known taxonomy (Saeed *et al.*, 1989);

. have a known embryology (Howe, 1987);

. may react similarly to other pseudomugilid species that are found in restricted ranges. Pseudomugilids are a fairly homogeneous group.

The present study comprised two main suites of experiments (Tank Experiments I and II) to verify and elucidate the nature of the impact of *G. holbrooki* on *P. signifer* under controlled conditions. In addition, two behavioural observation studies (I and II) were performed.

The first major experiment was devised to answer the question:-

1. Does the presence of *G. holbrooki* present a threat to the survival and growth of *P. signifer*? It has been suggested that *G. holbrooki* may affect the rate of growth of native fish (Lloyd 1989). For this reason data were collected on lengths and weights of *P. signifer* before and after culture in the presence or absence of *G. holbrooki*.

From the first experiment, two more questions arose which formed the basis for the second major experiment:-

2. Does the presence of *G. holbrooki* affect the fecundity of *P. signifer*?

3. Is food a limiting factor in the decrease in numbers and failure of growth of *P. signifer* that was observed in experiments when they were housed with *G. holbrooki*?

4.2 Materials and Methods.

4.2.1 Tank Experiment I - The impact of *G. holbrooki* on the survival, growth and reproduction of *P. signifer*.

4.2.1.1 Duration of experiment. The experiment was carried out between late winter (August 1992) to mid summer (February 1993), to cover the main breeding season for both species.

4.2.1.2 Tank set-up. Eight tanks were constructed in the open air from 100 litre, half-cylindrical containers of food grade black polyethylene. The diameter was 42cm with a length of 80cm and filled to a depth of 19cm. The large surface area-to-volume ratio provided a substantial area for gas exchange. They were placed on a trestle in a row with each tank slightly inclined towards a screened overflow device,

allowing excess water from rainfall to flow out without loss of fish.

In late August they were filled with water from 2,700 litre ponds that had been established three months earlier (June) to equilibrate. In each pond, 500 grams of straw had been placed to inhibit the growth of algae (Barrett 1991). Well washed river sand was used as a substrate. Plant materials, including *Taxiphyllum barbieri*, *Ceratopteris* sp. and *Lemna* sp. were distributed equally among the test tanks, to be used for cover and spawning by the fish.

The test tanks were screened with wire mesh to prevent predation by birds and for security. The test tanks were allowed to equilibrate for three months prior to introducing the fish for the study (Zieris, Feind and Huber 1988).

4.2.1.3 Water quality analysis.

Water quality parameters were measured in October immediately prior to fish introduction, in December, mid-way through the experiment, and in February prior to harvest. Samples were taken for the measurement of Ph, temperature, dissolved oxygen, biological oxygen demand (BOD), ammonia, nitrate and hardness. Formalised 350ml water samples were also collected at the beginning of the experiment and at harvest for phytoplankton and zooplankton density analyses.

pH. pH was determined with a SCAN-2 pH Tester (Activon Scientific Thornleigh NSW Australia).

Temperature. Water temperature was measured in degrees celsius to one decimal place with a thermometer.

Dissolved oxygen. Dissolved oxygen concentration was determined using a modification of the Winkler method (Strickland and Parsons, 1972).

Biological oxygen demand. The biological oxygen demand (BOD) assesses the quantity of oxygen used for respiratory activities by microorganisms (Gaudy, 1972).

Dissolved oxygen concentrations for the BODs were determined with the modified

Winkler method (Strickland and Parsons, 1972). Duplicate initial samples were taken after saturating the sample by shaking a two litre sample in a five litre container for five minutes. Two measurements were then made. Duplicate oxygen saturated samples were incubated in BOD bottles at 20°C in the dark for 5 days before the final concentration of dissolved oxygen was determined. The BOD_5 was calculated by subtracting the final dissolved oxygen concentration from the initial concentration.

Ammonia. The total ammonia concentrations of duplicate water samples were measured chemically using a modification of the method of Strickland and Parsons (1972). The water samples were treated with alkaline citrate, sodium hypochlorite, and phenol in the presence of sodium nitroprusside. The blue indophenol formed was measured spectrophotometrically. In the present study, the method of Strickland and Parsons (1972) was modified. The volume of sample taken for analysis was one tenth (5ml) that used by these authors. The analysis was carried out in lidded vials. Accordingly 0.2ml of phenol solution, 0.2ml of sodium nitroprusside solution and 0.5ml of oxidising solution was added by micropipette in sequence with stirring between additions. Standards were made up in milli-Q water to the concentration of 0, 60, 120, 240, 480 and 960 μg/L ammonia as N. The samples and standards were allowed to stand at room temperature for an hour before measurement at 640nm in a spectrophotometer (Ultrospec II, LKB, Pharmacia, North Ryde, NSW). The sample concentrations were interpolated from a standard curve in μg/L ammonia as N. The un-ionised ammonia was calculated from tables of percent un-ionised ammonia in aqueous ammonia corrected for pH and temperature (Piper *et al.*, 1982).

Nitrate. Nitrates were determined on duplicate samples. They were measured chemically with a Segmented Flow Analyser (SFA) (SkalarScan System Model 400). It used a standard automatic colorimetric procedure with a dual path matrix photometer which compensated for background interferences. The concentration range was between 1-50 μg/l. Where necessary samples were diluted to fall within this range. A cadmium column was used to reduce nitrate to nitrite. The colour

change on addition of the chemicals (1% sulphanilamide in 10% HCl and 0.1% N-1-naphthyl ethylenediamine dihydrochloride) was measured colorimetrically.

Total hardness. Total hardness was measured in duplicate samples using an EDTA titrimetric method (APHA, 1992). In this method, ethylene diaminetetraacetic acid and its sodium salts (EDTA) form a chelated soluble complex when added to a solution of certain metal cations. The indicator Erichrome Black T was used in a buffered sample (Ammonium Buffer pH 10) producing a wine red colour. When .01M standard EDTA solution was used as a titrant, it complexed with the calcium and magnesium turning the solution blue. Magnesium must be present to reach an appropriate end point (APHA, 1992) and therefore a small quantity of complexometrically neutral magnesium salt of EDTA was added to the buffer.

Invertebrates and phytoplankton. One sample was taken from each tank, to determine invertebrate and phytoplankton abundance, at the beginning and end of the experiment. Samples of 350 ml tank water were collected by collecting seven subsamples with a 50ml scoop which was rapidly dipped at a number of points on the surface of the tank. *P. signifer* are known to be surface feeders (Booth *et al.* 1985). The subsamples from one tank were pooled and preserved with formalin to give a 5% solution. The samples were allowed to settle over several weeks. The supernatant was carefully taken off leaving 15ml for analysis. The solid scoop method prevents concentration of organisms during collection and the rapid dips minimises the escape of faster moving organisms (de Bernardi, 1984).

Large invertebrates. The 15ml concentrated sample was poured into a plastic petri dish and dominant species of invertebrates were selected for counting. Mosquito larvae, midge larvae, *Collembola*, cladocerans and copepods were identified and counted under a dissecting microscope. As the sample was concentrated from a known volume of water and the entire sample counted the number of organisms counted were converted to give the number of organisms per litre.

Small invertebrates and phytoplankton. After the large invertebrates were analysed the 15 ml concentrated sample was centrifuged for five minutes at 500rpm.

13ml of supernatant was removed leaving 2ml for analysis. 1ml of this sample was analysed using a Sedgewick-Rafter cell with a total volume of 1 ml. Twenty random fields of view were counted for dominant species of small invertebrates and phytoplankton using an Olympus Compound Microscope Model E. The number of each type of organism was corrected for the volume from which the sample was concentrated and for the size of the field of view to give the number of organisms per litre.

4.2.1.4 Fish collection. Fish were collected from the estuarine reaches of Deep Creek in October 1992 and acclimated to freshwater over a period of ten days. Pond water was used for this acclimation. Gee (1988) successfully acclimated *P. signifer* from seawater to freshwater in less than seven hours. These fish are proven euryhaline from other studies as they can regulate their body fluid concentration when exposed to either hypo- or hyper-osmotic waters (Gee, 1988).

4.2.1.5 Fish measurement. Prior to introduction into the tanks the fish were measured for total length and standard length. They were also sexed. The males were identified by colour at this time of year (Chapter 3). Measurements were made with a measuring tube which maintained the fish in water (Litvak, 1983). This method was adopted to prevent the high death rates frequently associated with prolonged handling and exposure to air (Ricker, 1975).

Weights were interpolated from a weight curve derived from length/weight data collected from fish from the same population as those used in experiments, again to guard against high mortalities which occurred when fish were weighed prior to use in pilot experiments (see Appendix II). Statistical analysis of weights of test *P. signifer* individuals was performed at harvest.

4.2.1.6 Ratio and stocking numbers. Since it was not practical to test a wide range of densities and ratios of fish species and, indeed, unnecessary in determining whether the presence of the exotic species resulted in an adverse impact, a stocking ratio mimicking that in the field was used. In addition the ratio of 2:1 female to male was used which reflected that in the field (Table 4.1).

Meffe (1985) in his coexistence experiments with *G. affinis* and *Poeciliopsis*

occidentalis (similar sized fish to the ones used in this study) used a similar stocking density ratio. Krumholtz (1948) in a study of over 30,000 *Gambusia affinis* carried out from 1939-40 obtained a sex ratio of 175 females to 100 males.

In this present experiment a total stocking density of 18 individuals in 100 litres was used. On the basis of previous observations (unpublished data) it was considered that a lower stocking number may not have led to successful breeding. Because of the schooling habits of these species observed, natural densities of both these species of fish vary widely from a few individuals to over a thousand per m^3. The fish can be found in areas from wide expanses of water, frequently in the shallows, down to pot holes of very small volume.

Table 4.1 Numbers of Male and Female *P. signifer* caught at a variety of sites from 1985-1993, with sex ratios (female to male).

Date	Site	Male	Female	Ratio
16/2/85	Lane Cove	10	22	2.2
17/2/85	Deep Creek	3	8	2.7
19/10/85	Deep Creek	5	11	2.2
19/10/85	Lane Cove	8	17	2.1
27/1/86	Deep Creek	4	5	1.3
28/1/86	Lane Cove	47	25	0.5
20/9/93	Deep Creek	16	31	1.9
25/9/93	Deep Creek	31	53	1.7
12/93	Mooney Mooney	44	106	2.4
12/93	Hornebush	15	9	0.6
12/93	Lane Cove	30	51	1.7
12/93	Deep Creek	13	33	2.5
Mean±SE				1.8±0.2

4.2.1.7 Design. The tanks in the first experiment were set up in the following manner:- Four replicate control tanks containing 6 male and 12 female *P. signifer*.

Four replicate experimental tanks each containing :-

3 male and 6 female *P. signifer* = 9 fish.

3 male and 6 female *G. holbrooki* = 9 fish.

Total = 18 fish.

Total number of fish used in the experiment :-

36 male and 72 female *P. signifer*.

12 male and 24 female *G. holbrooki*.

Fig. 4.1 shows a diagrammatic representation of the test tanks, randomly assigned to their position in the series. Hence forth in this dissertation the term control tank refer to those stocked with *P. signifer* only. The experimental tanks refer to tanks which were stocked with both *P. signifer* and *G. holbrooki*.

4.2.1.8 Fish introduction and harvest. As the fish were measured, they were assigned to 5 litre containers filled with tank water. A few drops of methylene blue were added to prevent fungal infection after measurement (Leggett and Merrick, 1987).

The fish were measured and put into containers labelled 1-8. Each additional fish was assigned to a container in numerical order until the required number of each sex and species were obtained. The fish that were to be used for control and experimental treatments were randomly assigned to tanks.

Before release into the test tanks the containers were floated on the surface of the water of the tanks for 20 minutes to allow temperature equilibration. Dead fish were replaced in the first two weeks of the experiment and thereafter were not replaced.

The fish were maintained in the tanks from October 1992 to February 1993. This covered the breeding period of both species of fish. The fish were not fed after introduction to the tanks. They fed on material that fell into or grew in the tanks. At harvest the tanks were completely emptied with a 1 litre container and fish were netted out. They were identified, measured, sexed, weighed and counted. Fig. 4.2 shows the condition of the tank ecosystems prior to harvest.

4.2.2 Tank Experiment II - Impact of *G. holbrooki* on the fecundity of *P. signifer*, with and without augmented food supply.

The aims of this experiment were to determine the impact of *G. holbrooki* on the fecundity of *P. signifer*, and in addition to determine if food is a limiting resource in the lack of growth of *P. signifer* when housed with *G. holbrooki*.

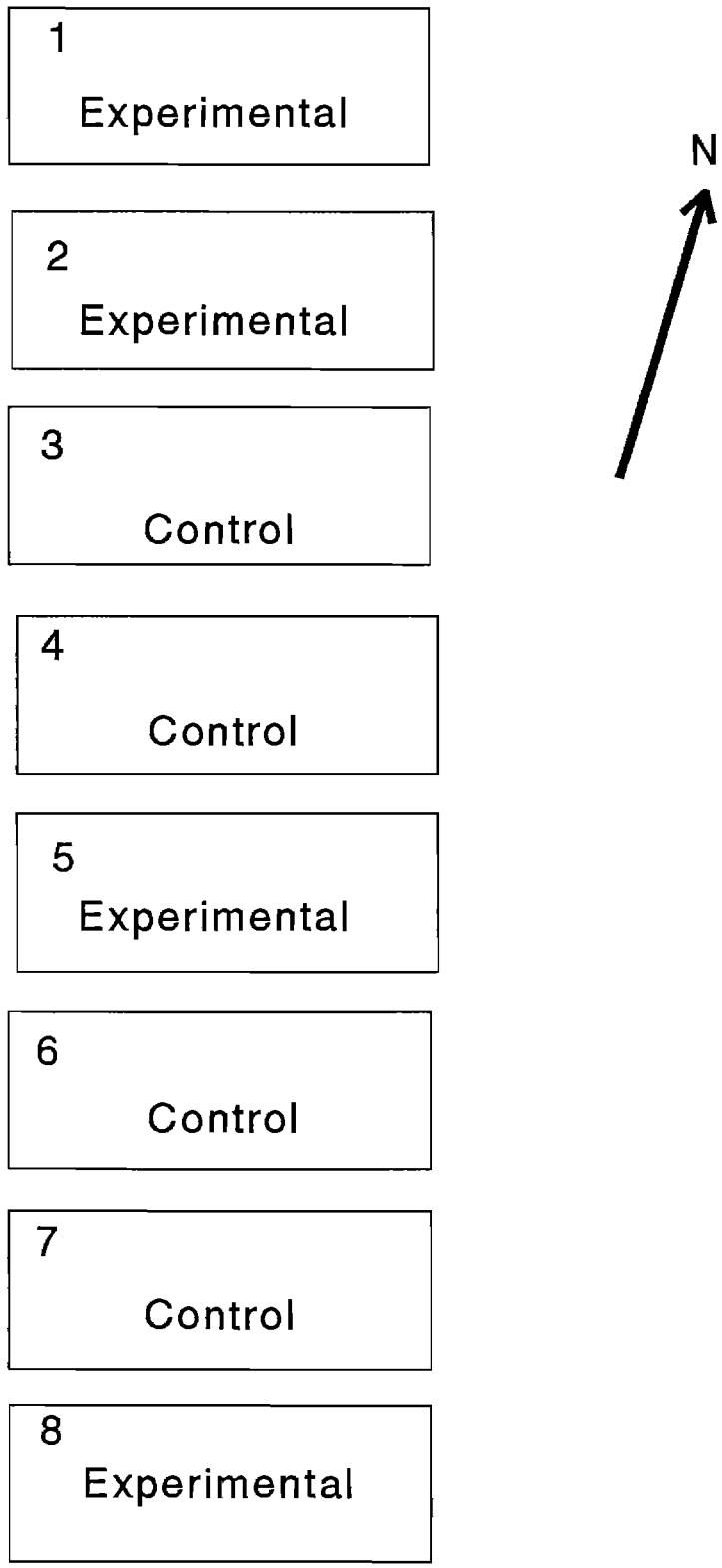


Figure 4.1 Diagrammatic representation of the random allocation of treatments. Control ponds contained 6 male and 12 female *Pseudomugil signifer*. Experimental ponds contained 3 males and 6 females each of *P. signifer* and *Gambusia holbrooki*.

Figure 4.2 The eight tanks used in Experiment I prior to harvest in February, 1993 (The tank in the foreground had been harvested).

4.2.2.1 Duration. The experiment was carried out between September and the end of December 1993.

4.2.2.2 Tank set-up. Sixteen tanks were set up in the same manner as in the first test tank experiment. Fig. 4.3 shows the condition of the experimental tanks during their establishment. They were filled with aged pond water (from previously established 2,700 litre ponds), with washed river sand as a substrate and plant material (*Lemna* sp. and *T. barbieri*) in September and allowed to equilibrate for 1 month before the introduction of fish.

4.2.2.3 Water quality analysis. Water samples were collected in duplicate prior to the introduction in October and in triplicate (to improve precision) in December at harvest.

4.2.2.4 Fish collection. The fish were collected in late September from Deep Creek and acclimated to freshwater over a period of 10 days.

4.2.2.5 Fish measurement, stocking ratio and sex ratios. The fish were measured in the same manner as described in the first outside tank experiment (Section 4.2.1). The experimental design was consistent with the first experiment. The same sex ratios and fish species numbers were used as in the first experiment. Fish were randomly assigned to the tanks as in the first experiment. There were 8 control tanks with 6 male and 12 female *P. signifer* and 8 experimental tanks with 3 male and 6 females of both species in each tank respectively. The total number of fish used for this experiment was 72 male and 144 female *P. signifer* and 24 male and 48 female *G. holbrooki*.

4.2.2.6 Fish introduction and harvest. Fish were introduced into the tanks in October 1993 in the same manner as described in the first tank experiment. Dead fish were replaced in the first two weeks of the experiment and thereafter not replaced.

Figure 4.3. The establishment of the sixteen tanks used in Tank Experiment II in September, 1993. The samples were analysed as described in the first experiment.

In this experiment 4 control and 4 experimental tank treatments were kept in the same manner as in the first tank experiment, that is, with no additional feeding. A further 4 control and 4 experimental tank treatments were provided with supplementary feeding of 5g of mosquito larvae, cladocerans, brineshrimp, or Tetra Min Staple commercial dried food each week. The different foods were supplied to give variety in the diet. The type of food supplied at any one time was dependent on availability (live food being given preferentially).

The fish were harvested at the end of December 1993, rather than in February as in the first tank experiment. The reasons for this were twofold:-

a) As this is a fecundity study, fish must be harvested at the peak of their breeding cycle. Field results (Chapter 3), had indicated that the ovaries in *P. signifer* were most active in December and decreased in February.

b) Since the intention was to compare these fecundity results with those in the field fecundity study (Chapter 5), simultaneous collection in December was again indicated.

At harvest the fish were identified, measured, sexed, weighed and counted as in the first experiment. In addition, the gonads were dissected out after anaesthetising the fish in 0.2g/l methane sulphonic acid (MS222). The gonads were weighed. Oocytes larger than 0.52mm in female *P. signifer* ovaries were counted as an estimate of fecundity (see Table 3.1). The developing embryos were also counted in female *G. holbrooki*.

4.2.2.7 Gonadosomatic index. The gonadosomatic index was calculated by dividing the weight of the gonad by the total weight of the fish.

4.2.2.8 Statistical analysis. Statistical analysis was carried out separately on the males, females and juveniles on both species of fish. A summary of analysis used in this series of experiments is presented in Table 4.2.

4.2.3 Laboratory behavioural studies.

The purpose of these behavioural studies was to observe the behavioural

interactions (possible interference competition or interspecies aggression) of the two species. In the out-door tank experiments it was extremely difficult to observe interactions as the tanks were opaque and observations could only be made from the surface, which was obscured by reflections and plant material (Fig. 4.2 and 4.3). No behavioural data were obtained from those experiments. The observational studies, in contrast, were conducted in glass aquaria. The fish could therefore be observed from all sides.

Table 4.2. Methods used for measurements performed on fish in tank experiments I and II.

Experiment	Comparison of	Test	Reason
I & II	Numbers	One way ANOVA with Tukeys post test	Compare treatments at harvest
I & II	Survival	1-tailed t-test. The number of mature adults could only decline	Introduction versus harvest
I & II	Proportion remaining at harvest	Non parametric Mann Whitney (one tailed) (I) Kruskal Wallis analysis of variance with Dunns post test (II)	Numbers at harvest were converted to a proportion of those at introduction.
I & II	Standard length Total length Weight	One way ANOVA with Tukeys post test	Compare treatments at harvest
I & II	Standard length Total length Weight	2 tailed t-test.	Introduction versus harvest. The mean TL, SL or weight could rise or fall.
II	Fecundity Ovarian weight	Non parametric Kruskal Wallis ANOVA with Dunns post test	SD highly variable.
II	Testis weight	One way analysis of variance with Tukey Kramer's post test	*P. signifer* samples only, terminal samples
II	Testis weight	Unpaired t-test	*G. holbrooki* in 2 treatments only
II	GSI	One way ANOVA (male) Kruskal Wallis non-parametric ANOVA (female)	Male *P. signifer* Female *P. signifer* had variable standard deviations Compare treatments at harvest
II	GSI	Mean difference tested by unpaired t-test	*G. holbrooki* in two treatments only
I & II	Fish survival and growth parameters versus water quality and invertebrates	Linear regression	Relationship between fish survival and growth and water quality and invertebrates

4.2.3.1 Behavioural observation I: Interactions of *P. signifer* housed with and without *G. holbrooki*.

This observational study was designed to mimic that of the first test tank experiment. Impacts measured in the out-door experiment could be complemented by behavioural observations in the laboratory. These studies were needed to look for behaviour which might relate to effects on growth and development found in *P. signifer* in the outdoor study. Without the behavioural study it would be difficult to determine that any differences in length and weight or fin nipping could be attributed in any way to *G. holbrooki*. It was also of interest to determine whether different social behaviours could be observed when *G. holbrooki* were housed with *P. signifer*.

The behavioural study was set up in 48cm x 58cm x 37cm high 100 litre aquaria (similar volume as the test tank experiment) with glass sides. Well-washed river sand was used as a substrate. *Taxiphyllum barbieri* was provided for spawning. Rocks were placed in the aquaria to provide shelter for the fish. The water was filtered with a box filter. Water changes of 5 litres were made every three weeks with aged Sydney tap water.

Two aquaria subjected to natural temperature conditions were used. They were set up using the same water as for the outside tank experiment and allowed to equilibrate for the same time period.

Water Quality. Water quality analysis was carried out as described in Section 4.2.1. However, BOD and dissolved oxygen concentrations were not measured because the aquaria were aerated and filtered.

Fish measurements, ratio and stocking numbers. Lengths were measured and weights interpolated on introduction as for the tank experiment. One aquarium acted as the control treatment and had 6 male and 12 female *P. signifer*. The other aquarium housed the experimental treatment and housed 3 male and 6 female *P. signifer* and 3 male and 6 female *G. holbrooki*.

Design. Unlike the first test tank experiment the fish in this behaviour study were fed daily as no natural food was available. The fish were fed 0.2 g of Tetra Min Staple Food daily and supplemented with 0.5 g frozen brineshrimp weekly and live mosquito larvae (equal amounts to each aquaria) when available.

Fish behaviour and general observations. Observations were made for thirty minutes in the morning, 8.00 to 8.30 am, and thirty minutes in the afternoon from 5.00 to 5.30pm. During the assigned observation time an assessment was made weekly as to whether:-

. any fin nipping took place between species or within species;

. either species dominated whilst feeding;

. aggressive behaviour.

These behavioural interactions were recorded as positive or negative, for each species or between species. The sex of any offending individual was similarly recorded. The general condition of the fish was also assessed. An assessment of spawning behaviour and colour development in the case of *P. signifer* males was recorded. Searches were made weekly for *P. signifer* eggs in the plant material. The aquaria were also examined for any fish larvae.

4.2.3.2 Behavioural observations II. Interactions between adult *P. signifer* and *G. holbrooki* and larval *P. signifer*.

The aim of this experiment was to determine the interactions of adult *G. holbrooki* and *P. signifer* with the young of *P. signifer*. In particular it was hoped to discover whether adults of the two species preyed on larvae of *P. signifer*.

Design. Three adult *P. signifer* and *G. holbrooki* in a 1:2 male to female sex ratio were introduced to 6, one-week old *P. signifer* larvae. The fish larvae were first established in a 25cm x 48cm x 30cm height all-glass aquarium for a week. Adequate hiding places were provided.

Observations. One week after introduction the *P.signifer* larvae were observed to be

completely acclimated to their new environment and all appeared to be healthy as assessed by their swimming and intake of food. Observations were made on the survival of these larvae after introduction of the adults. The observations commenced as soon as the adult fish were introduced and continued until the demise of all of the larval young.

4.3 Results.

4.3.1. Tank experiment I - Impact of *G. holbrooki* on the survival, growth and reproduction of *P. signifer*.

4.3.1.1 Water quality.

The water quality results are presented first, to show homogeneity between treatments and that adequate water quality was maintained through the experiment. Details of the specific results are then provided on survival rates, and growth parameters are presented on *P. signifer* then *G. holbrooki*.

pH (Fig 4.4A). Prior to introduction of the fish, the tank water was considered to have an acceptable range (8.1-8.5) for fish according to Langdon (1988) and Alabaster and Lloyd (1982). The pH, midway through the experiment in the control tanks appeared to be higher than that at the beginning or at harvest. It was not however, statistically significant (Kruskal Wallis non-parametric ANOVA). No difference was found between the median pH in control and experimental tanks (Mann-Whitney test).

Temperature (Fig 4.4B). Water temperatures in both control and experimental tanks fell within the range in which *P. signifer* have been found in the field (Merrick, 1984). The temperatures in both control and experimental tanks were not significantly different at introduction, midway and harvest (unpaired t-test). There was a significant increase in temperature through the experiment, consistent with increasing ambient temperature. ($P<0.0001$, one-way ANOVA, both control and experimental tanks).

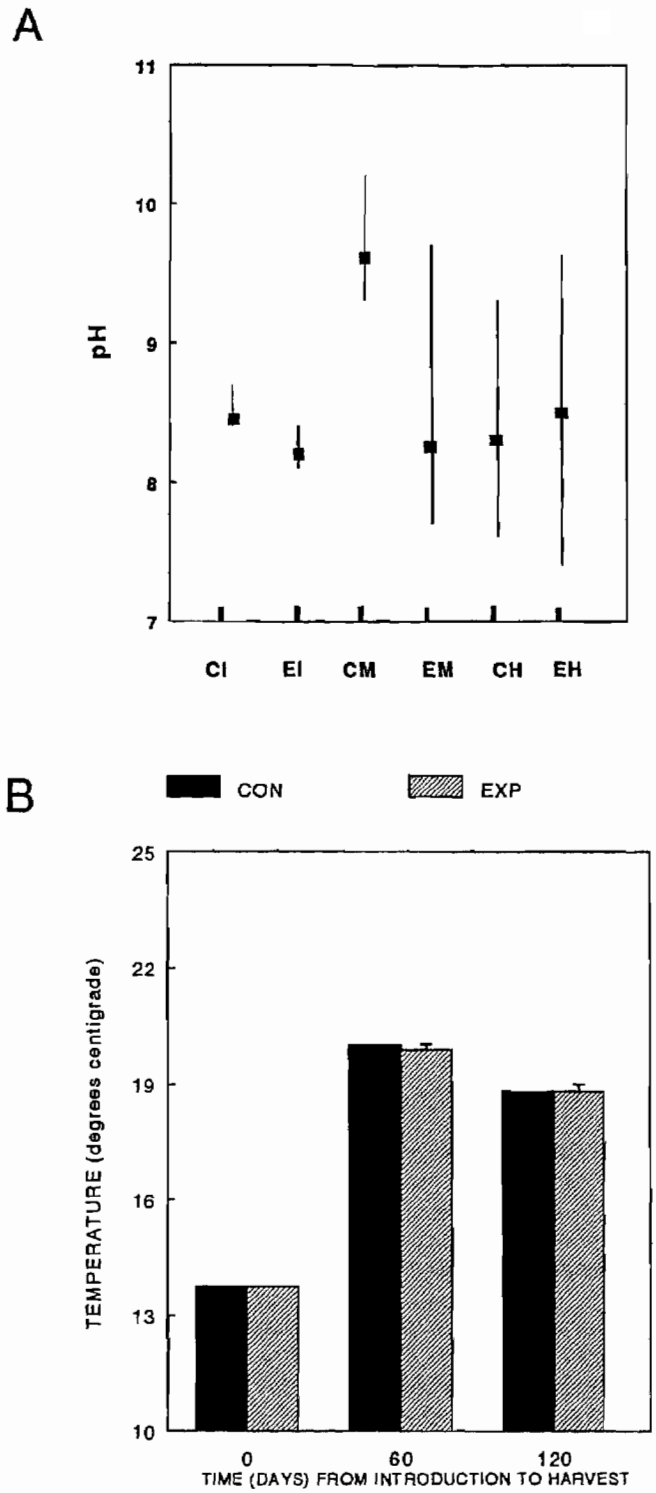


Figure 4.4 pH (range and medians n=4) (A) and temperature (mean ± SE n=4) (B) in tanks with *Pseudomugil signifer* only (CON) and tanks with *P. signifer* and *Gambusia holbrooki* (EXP) taken at the beginning, midway and harvest of the experiment.
CI, control introduction; EI, experimental introduction; CM, control midway; EM, experimental midway; CH, control harvest and EH, experimental harvest.

Dissolved oxygen (Fig. 4.5A). Dissolved oxygen levels declined significantly through the experiment in the control tank (P=0.0002, one-way ANOVA), but not in the experimental tanks. At introduction all tanks had dissolved oxygen levels within the range 9.0-10.0 mg/l (i.e. well above optimum levels). Midway through the experiment the dissolved oxygen level was above 5.8 with the exception of tank 1 (experimental) which was 3.0. At harvest tanks 1 and 2 (experimentals) had low dissolved oxygen levels of 0.8 and 0.6 mg/l respectively and tank 4 control had a dissolved oxygen of 3.0 mg/l. The numbers, and growth in length and weight of *P. signifer* may have been affected by this low dissolved oxygen. A 2-tailed t-test was performed to determine whether there were significantly less *P. signifer* and less growth in survivors in the two experimental tanks which had lower dissolved oxygen than the other two. No significant difference in the male or female survival was found between the tanks with low dissolved oxygen compared to the other two tanks. No statistical analysis could be performed on the length and weight of male *P. signifer* from experimental tanks with different dissolved oxygen levels at harvest as two tanks had only one fish and another had none. There was no significant difference between the tanks of differing dissolved oxygen in the length and weight of female *P. signifer*. The results obtained comparing the numbers, total length and weight between controls and experimentals can therefore be used with relative confidence. There was however, no significant difference in median dissolved oxygen levels between control and experimental tanks at introduction or harvest (Mann-Whitney test).

No correlation was found between dissolved oxygen at harvest and the proportion of *P. signifer* surviving to harvest. In addition, there was no correlation between dissolved oxygen and either weight or total length of *P. signifer* at harvest.

Biological oxygen demand (BOD) (Fig. 4.5B). BOD did not significantly change through the course of the experiment in either control or experimental tanks (one-way ANOVA). No significant difference was found in BOD between control and experimental tanks at introduction, midway or harvest (unpaired t-test).

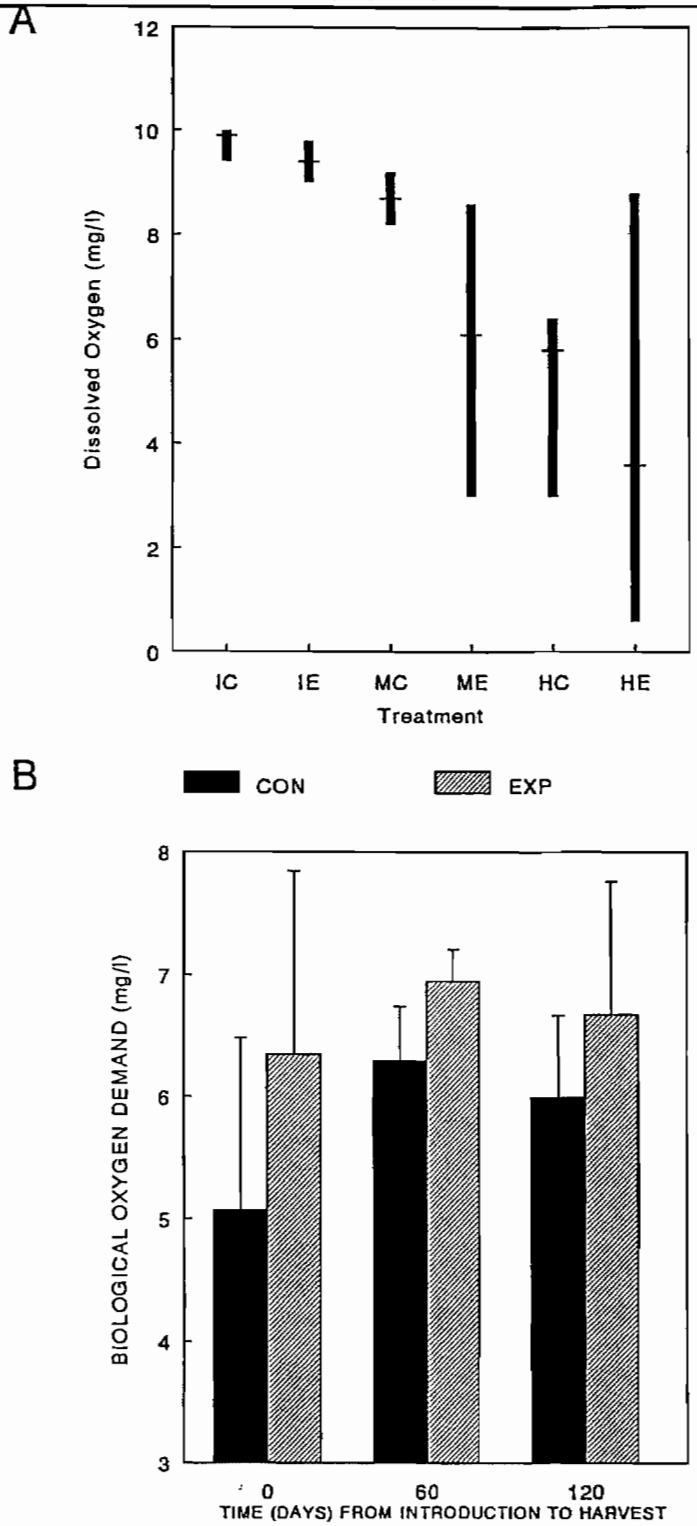


Figure 4.5 The dissolved oxygen concentrations (A) (median and range, n=4) and biological oxygen demand (B) (mean ± SE, n=4) of tanks with *Pseudomugil signifer* only (CON) and tanks with *P. signifer* and *Gambusia holbrooki* (EXP) taken at the beginning, midway and harvest of the experiment.
IC, introduction control; IE, introduction experimental; MC, midway control; ME, midway experimental; HC, Harvest control and HE, harvest experimental.

Un-ionised ammonia. Fig. 4.6A shows un-ionised ammonia (mean and standard error) at the introduction, midway through the experiment and at harvest in control and experimental tanks. Un-ionised ammonia levels did not significantly change through the course of the experiment in the experimental tanks (one-way ANOVA). In the control tanks un-ionised ammonia increased sharply midway through the experiment, but introduction and harvest values did not differ ($P<0.0001$, one-way ANOVA with Tukey-Kramer multiple comparisons test).

Un-ionised ammonia levels did not differ between control and experimental tanks at introduction and harvest but un-ionised ammonia levels midway through the experiment in control tanks were significantly elevated compared to the corresponding experimental tanks ($P=0.0073$, unpaired t-test). No significant difference was found in the un-ionised ammonia levels between the control and experimental tanks (unpaired t-test). The experimental ponds were not found to differ in the level of un-ionised ammonia from the initial measurement through to harvest. The control ponds were found to have a higher level of un-ionised ammonia mid way through the experiment (mean of 37 ± 4 μg/l) in comparison with the introductory (mean of 1.5 ± 0.7 μg/l) and harvest (mean of 0.8 ± 0.3 μg/l) measurements ($P<0.001$ one-way ANOVA with Tukey-Kramer multiple comparisons test).

Nitrate (Fig. 4.6B). No significant difference in nitrate levels was found at introduction, midway and harvest between control and experimental tanks (unpaired t-test). Nitrate levels significantly declined throughout the experiment in control ($P=0.0231$) but not experimental ponds (one-way ANOVA).

Hardness (Fig. 4.7). Langdon (1988) found that between 20-200 mg/l hardness is acceptable for fish culture. The hardness levels measured within the tanks fell within this range. There was no significant difference in hardness between control and experimental treatments at introduction, midway or harvest (unpaired t-test).

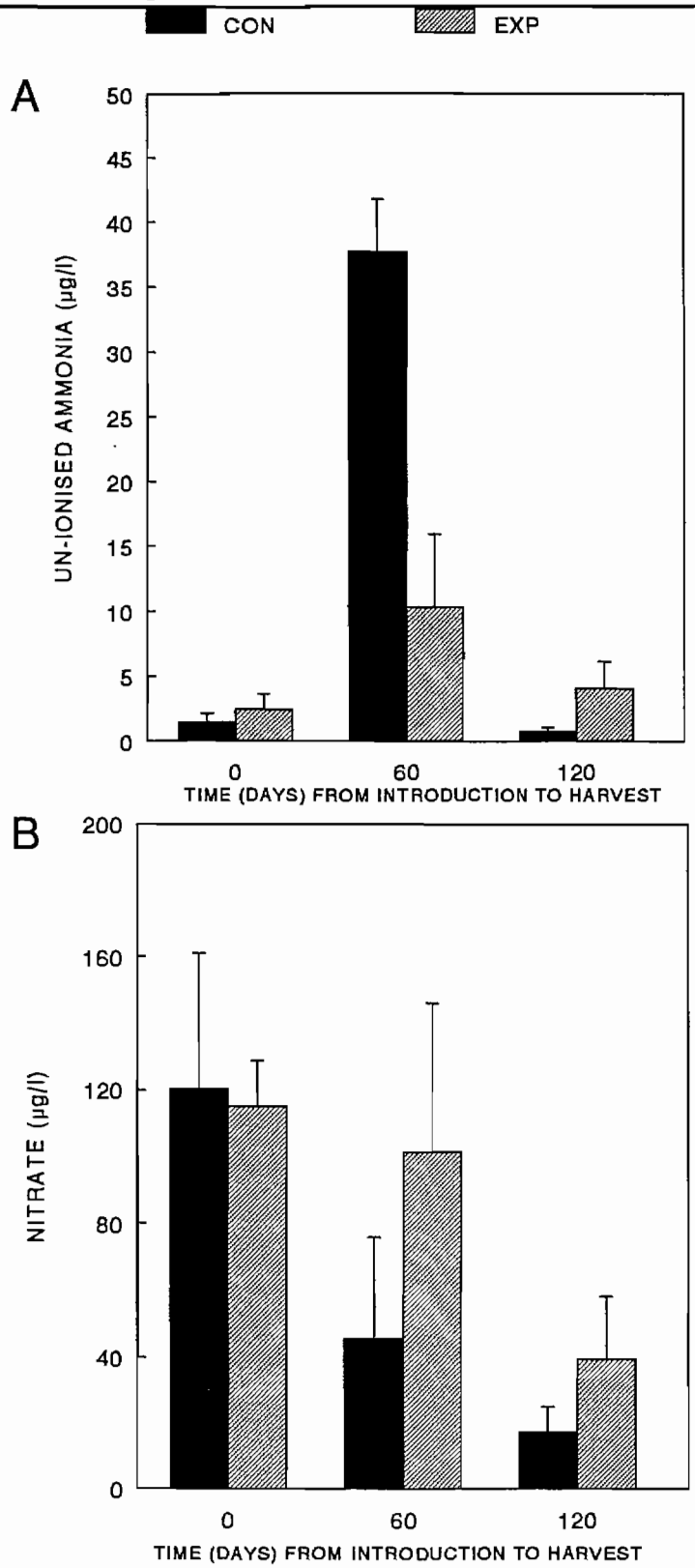


Figure 4.6 The un-ionised ammonia concentrations (A) and nitrate concentrations (B) (mean ± SE, n=4) of tanks with *Pseudomugil signifer* only (CON) and tanks with *P. signifer* and *Gambusia holbrooki* (EXP) measured at the beginning, midway and harvest of the experiment.

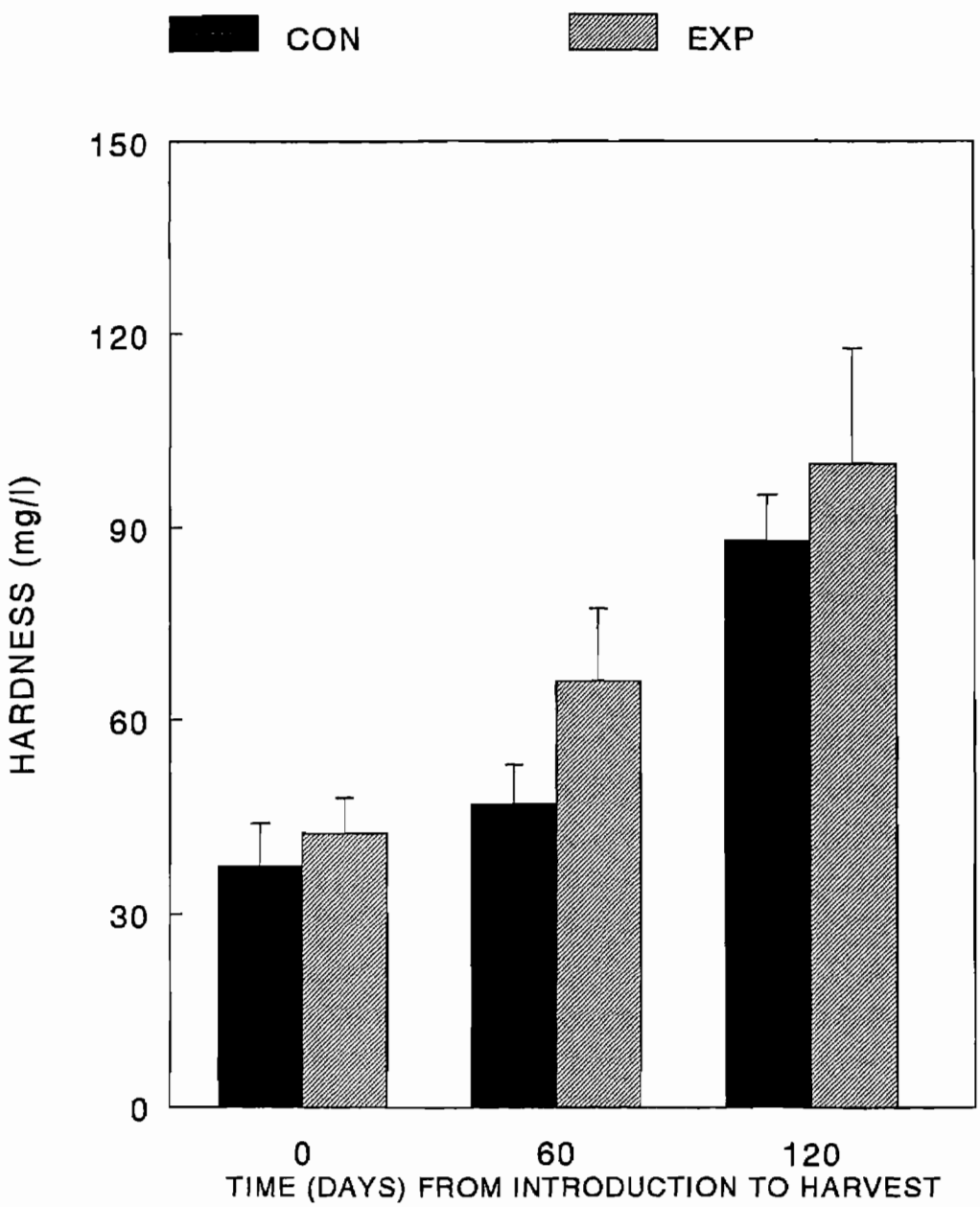


Figure 4.7 The hardness levels (mean ± SE, n=4) of tanks with *Pseudomugil signifer* only (CON) and tanks with *P. signifer* and *Gambusia holbrooki* (EXP) measured at the beginning, midway and harvest of the experiment.

Invertebrates and phytoplankton. The results of the counts of invertebrates and phytoplankton are presented in Table 4.3 and 4.4.

Mosquito larvae were present in all ponds prior to the introduction of the fish but none were found at harvest in either control or experimental ponds. At harvest however, there were relatively large numbers of rotifers which both species are known to eat (Gale, 1915 and Hurlbert and Mulla, 1981). The rotifers, cladocerans and phytoplankton had all increased in number by harvest.

Table 4.3. Abundance of invertebrates at the introduction and at harvest in control and experimental tanks (Mean number of organisms/litre ± standard error).

Treatment	Mosquito larvae	Copepods	Midge larvae	Rotifers	Copepod naupilii	Cladocerans
Introduction Controls	7.8±3.9	0.7±0.7	2.1±2.1	529±171	294±159	0
Introduction Experimentals	12.1±3.6	0	0.7±0.7	647±323	282±53	1.4±1.4
Harvest Controls	0	4.3±0.9	0.7±0.7	1,911±823	135±112	5.0±3.4
Harvest Experimentals	0	2.0±1.4	0.7±0.7	10,396±7,491	0	2.1±1.4

Table 4.4. Abundance of phytoplankton at the introduction and at harvest in control and experimental tanks (Mean number of organisms /litre ± Standard Error n=20).

Treatment	Algae 1	Algae 2	Algae 3	Algae 4	Algae 5
Intro Control	223±100	147±59	24,990±14,170	312±165	76±59
Intro Expti	176±71	223±129	5,880±4,762	265±194	29±18
Har Control	1,793±1,416	265±247	22,167±17,757	2,793±1,541	194±153
Har Expti	1,000±517	47±47	32,752±32,752	30,870±14,347	2,487±1,441

Algae 1 Phylum Chlorophyta Order Chlorococcales **Crucigenia** 75μm
2 Phylum Chrysophyta Chrysomonad 100μm
3 Phylum Chlorophyta **Chlorella** 10-15μm
4 Filamentous Chlorophytes (cells 75x20μm)
5 Phylum Chlorophyta **Oocystis** 40μm

4.3.1.2 Fish parameter measurements: *P. signifer.*

Survival (Fig. 4.8 A and B). In both sexes of *P. signifer* and in both treatments (controls and experimentals) the number of individuals surviving to harvest declined significantly from introduction (1-tailed single sample t-test). For male *P. signifer* only, the decline in proportion of individuals from introduction to harvest was greater in the presence of *G. holbrooki* ($P=0.0148$, 1-tailed Mann-Whitney test).

Total and standard length (Fig. 4.9, 4.10). The treatment groups had homogeneous variances at the commencement and at harvest of the experiment with respect to total and standard lengths (F statistic). Among males of *P. signifer* there was no significant change in total lengths from introduction to harvest in either control or experimental tanks. Likewise, no significant increase in standard length was found in male *P. signifer* from experimental tanks from introduction to harvest. However, in the control tanks there was an increase in standard length among male *P. signifer* from introduction to harvest ($P=0.019$, unpaired t-test). At harvest the total lengths and standard lengths of control male *P. signifer* were not significantly different from those of experimental male *P. signifer* (unpaired t-test).

In females very considerable increases in total and standard lengths were observed from introduction to harvest in the absence of *G. holbrooki* ($P<0.0001$, unpaired t-test). No significant increase in total length or standard length occurred in female *P. signifer* in the presence of *G. holbrooki*. At harvest the total lengths and standard lengths of control female *P. signifer* were significantly different from experimental female *P. signifer* ($P=0.002$ and 0.0369 respectively, unpaired t-test).

Weights (Fig. 4.11). In the absence of *G. holbrooki* both male and female *P. signifer* increased in weight from introduction to harvest ($P=0.0023$ and 0.0011, male and female respectively, unpaired t-test). No weight increase occurred when *P. signifer* were housed with *G. holbrooki*. Male controls were not significantly different in weight from experimental *P. signifer* males at harvest. However, female experimental *P. signifer* were significantly smaller than controls at harvest ($P=0.0021$, unpaired t-test).

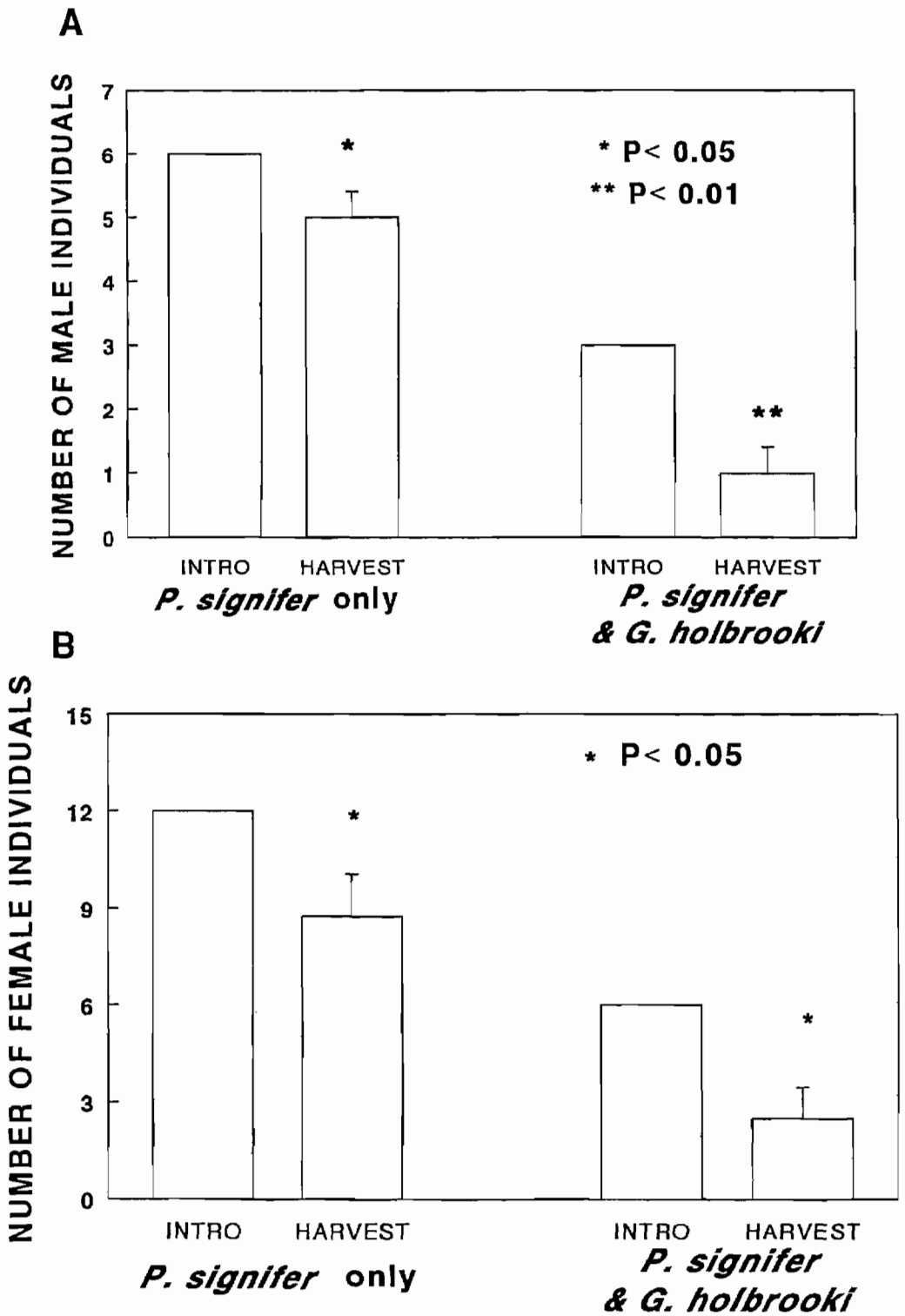


Figure 4.8 The mean number of individual (mean ± SE, n=4) male (A) and female (B) *Pseudomugil signifer* at introduction (INTRO) and at harvest in tanks with *P. signifer* only and tanks with *P. signifer* and *Gambusia holbrooki*.

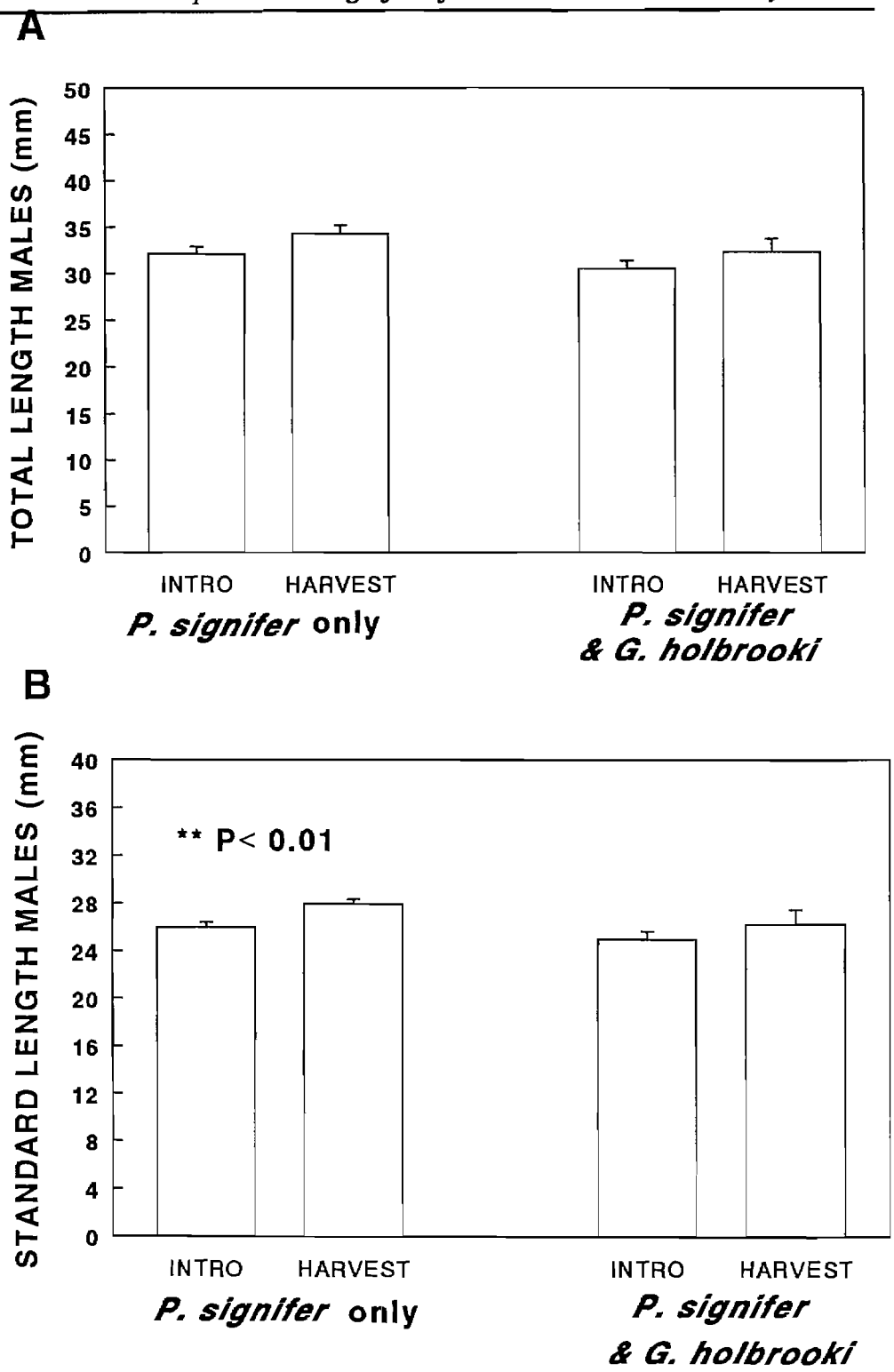


Figure 4.9 The total length (A) and standard length (B) of individuals (mean ± SE) of male *Pseudomugil signifer* at introduction (INTRO) and harvest in tanks with *P. signifer* only (Controls) and tanks with *P. signifer* and *Gambusia holbrooki* (Experimentals).
Control introduction, n=24; control harvest, n=20.
Experimental introduction, n=12; experimental harvest, n=4.

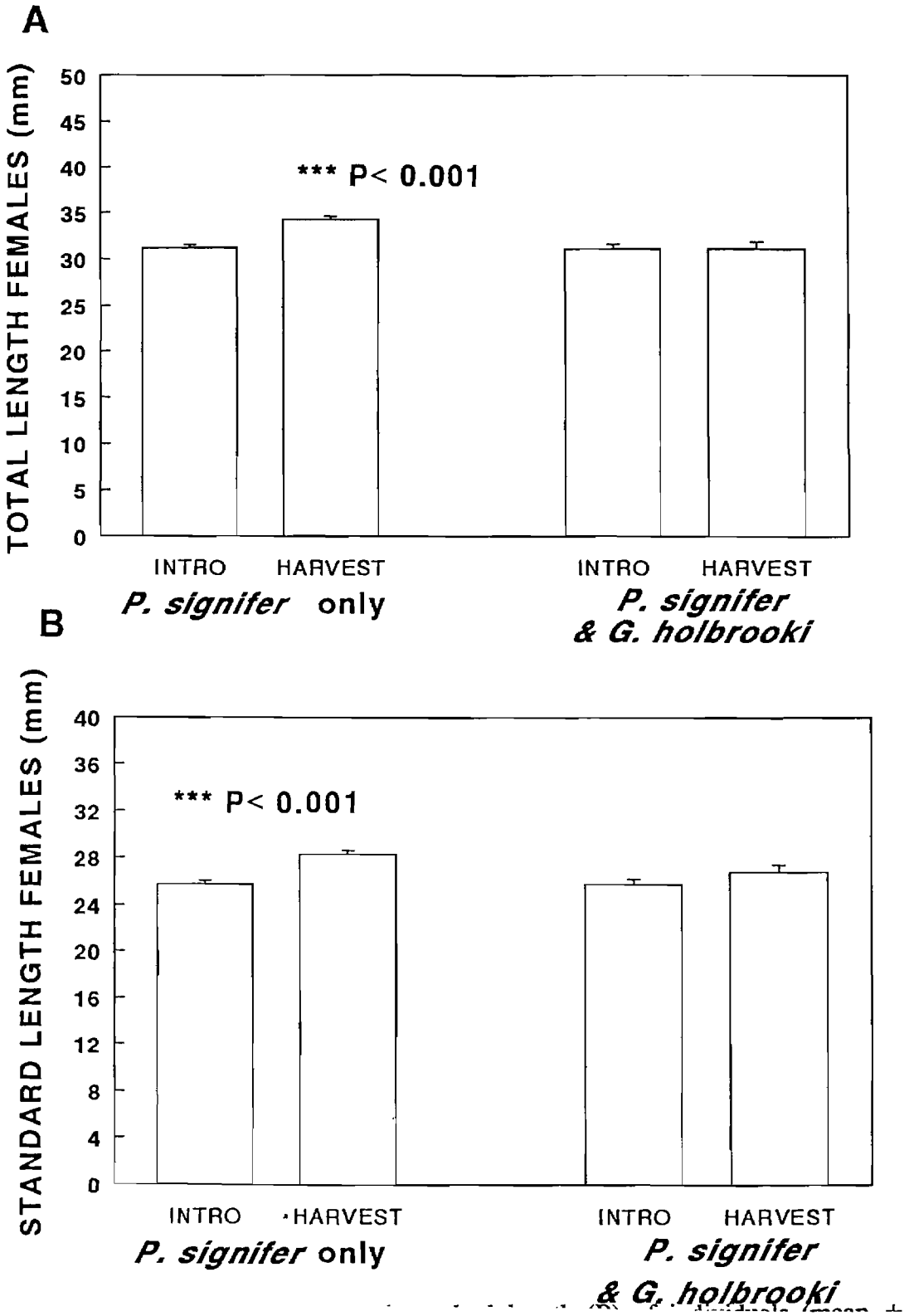


Figure 4.10 The total length (A) and standard length (B) of individuals (mean ± SE) of female *Pseudomugil signifer* at introduction (INTRO) and harvest in tanks with *P. signifer* only (Controls) and tanks with *P. signifer* and *Gambusia holbrooki* (Experimentals).
Control introduction, n=48; control harvest, n=35.
Experimental introduction, n=24; experimental harvest, n=10.

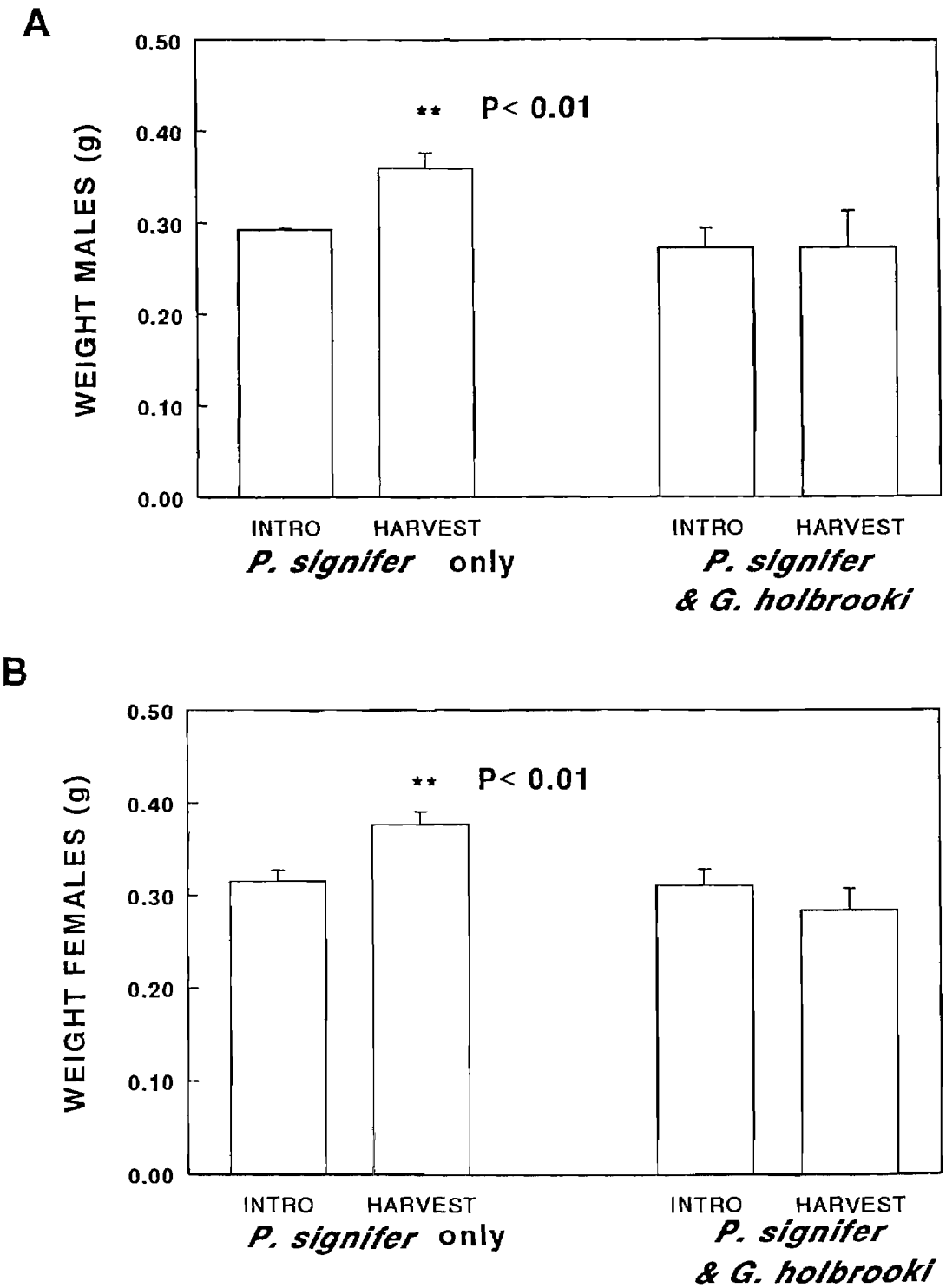


Figure 4.11 The weights of male (A) and female (B) individuals (mean ± SE) of *Pseudomugil signifer* at introduction (INTRO) and harvest in tanks with *P. signifer* only (Controls) and tanks with *P. signifer* and *Gambusia holbrooki* (Experimentals).
Males, control introduction, n=24; control harvest, n=20.
Experimental introduction, n=12; experimental harvest, n=4.
Females, control introduction, n=48; control harvest, n=35.
Experimental introduction, n=24; experimental harvest, n=10.

G. holbrooki.

Survival (Fig. 4.12). No significant difference was found in either sex, in survival numbers, from introduction to harvest.

Total and standard length (Fig. 4.13, 4.14). The distributions of total and standard lengths of *G. holbrooki* in the four experimental tanks had homogeneous variances at the commencement of the experiment (F statistic). No significant increases in total or standard length among males of *G. holbrooki* were found from introduction to harvest. There was an increase in both total and standard length from introduction to harvest in female *G. holbrooki* ($P=0.0001$, unpaired t-test).

At introduction the total lengths of females of *G. holbrooki* between tanks were not significantly different (one-way ANOVA). However, at harvest the total lengths of females between different tanks were significantly different ($P=0.0006$, one-way ANOVA). One tank contained very large females compared with individuals in other tanks at harvest. The presence of the group of large females did not affect the outcome of the experiment, as the outcome was similar if the results were considered with the tanks containing the largest and/or the smallest females removed ($P=0.0001$, unpaired t-test in each case).

All female *G. holbrooki* grew when maintained with *P. signifer*.

Weight (Fig 4.15). No growth in weight in males of *G. holbrooki* was found from introduction to harvest. However, females of *G. holbrooki* had significant growth in weight from introduction to harvest ($P<0.0001$, unpaired t-test). The weights at introduction were interpolated from a total length/weight curve (Appendix II). The weight of male *G. holbrooki* between the four experimental tanks at harvest were homogenous (one-way ANOVA). However, the weight data of females of *G. holbrooki* from the experimental tanks at harvest were not homogeneous. Despite this it is clear that female *G. holbrooki* did grow in weight in all tanks ($P<0.0001$, unpaired t-test).

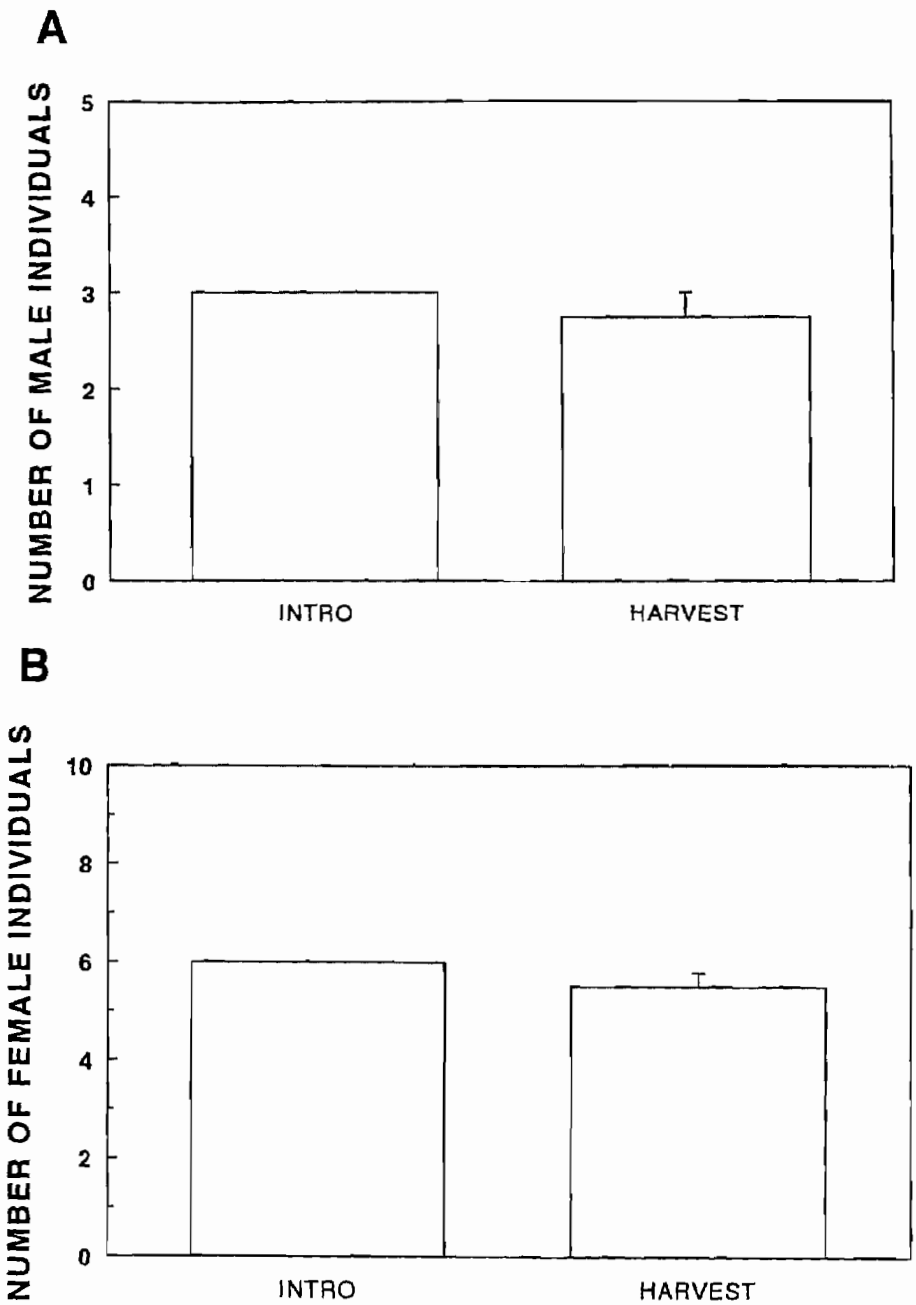


Figure 4.12 The mean number of individuals (mean ± SE, n=4) male (A) and female (B) *Gambusia holbrooki* at introduction (INTRO) and at harvest in tanks with *Pseudomugil signifer*.

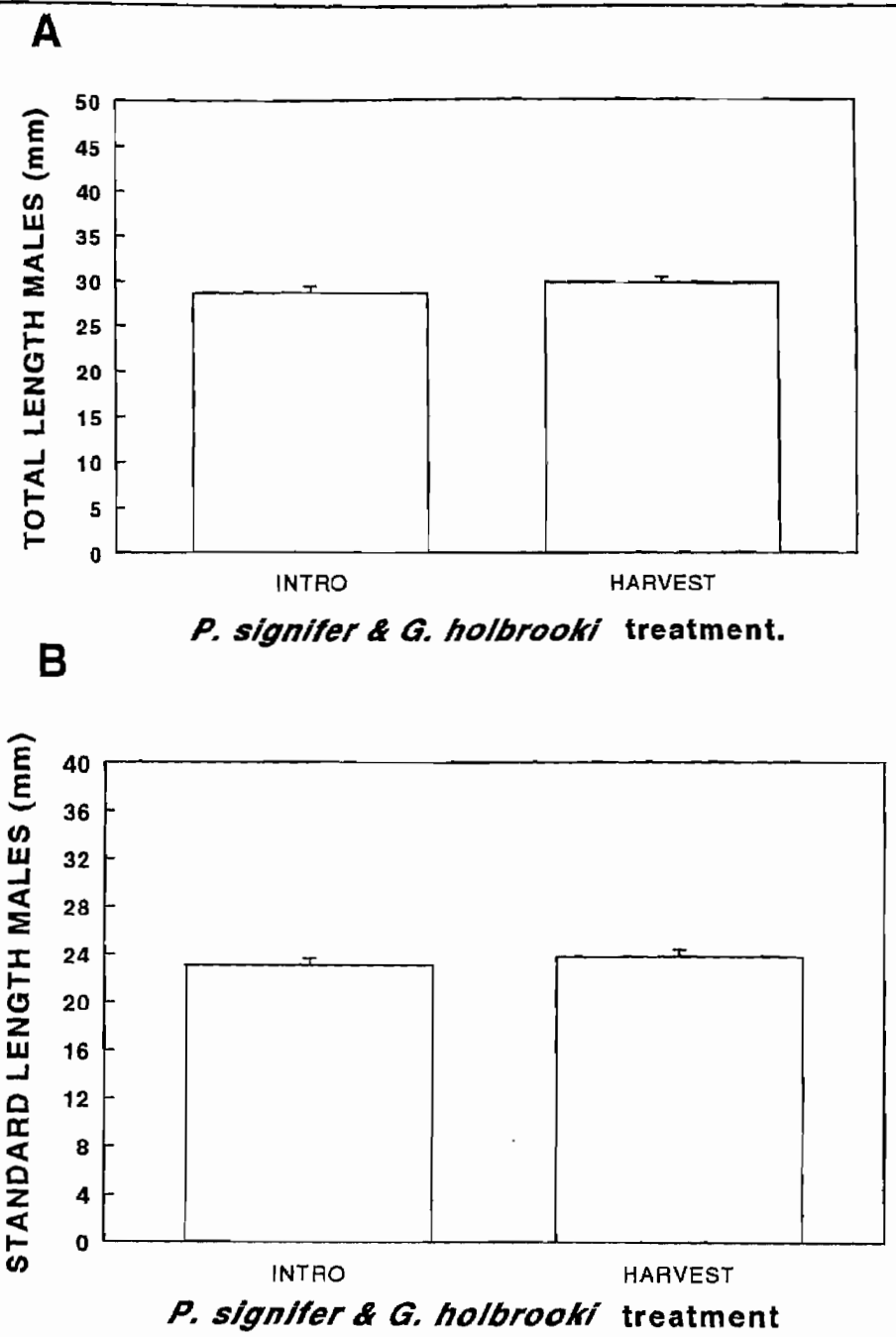


Figure 4.13 The total length (A) and standard length (B) of individual (mean ± SE) male *Gambusia holbrooki* at introduction (INTRO) and at harvest in tanks with *Pseudomugil signifer*.
Experimental introduction, n=12; experimental harvest, n=11.

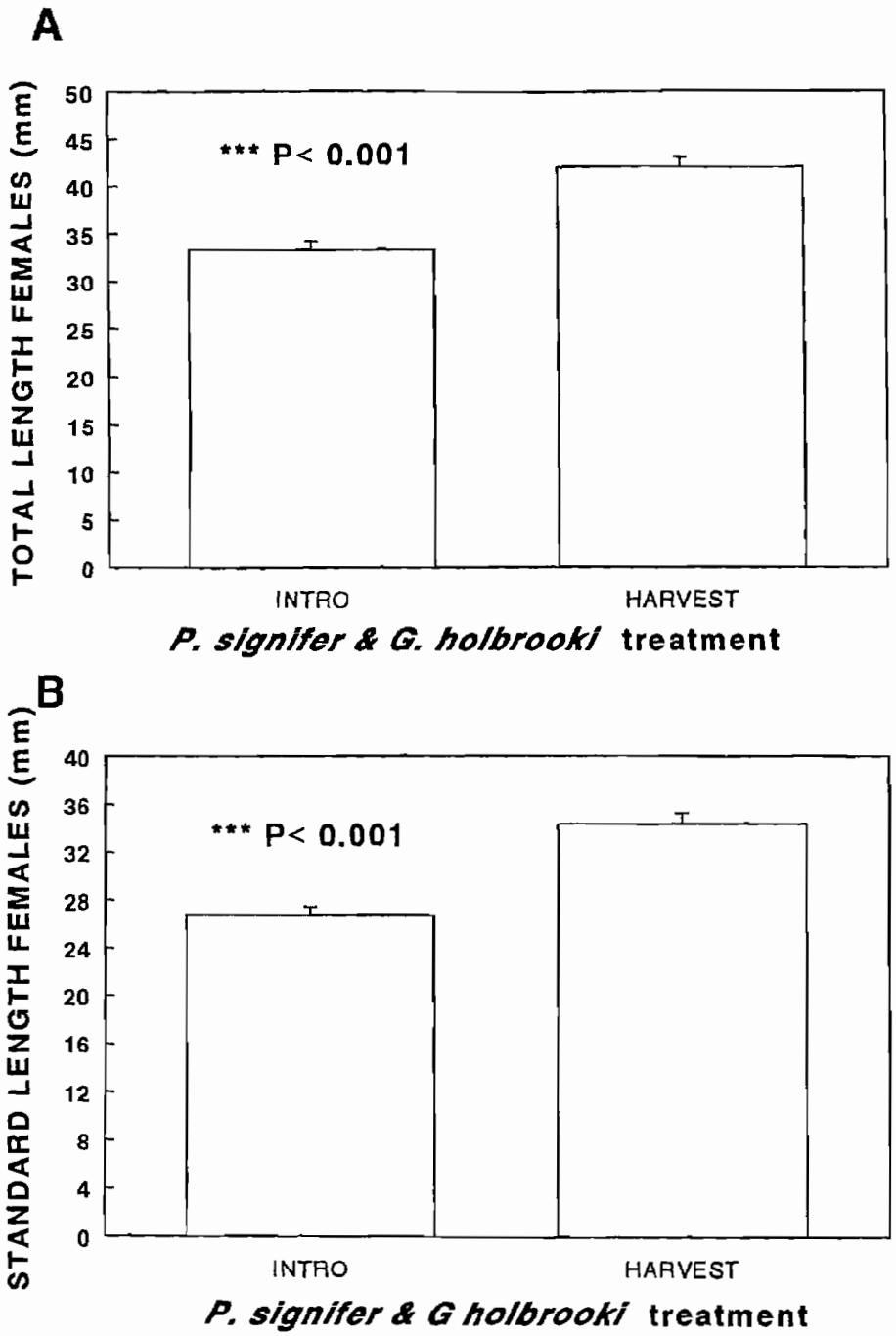


Figure 4.14 The total length (A) and standard length (B) of individual (mean ± SE) female *Gambusia holbrooki* at introduction (INTRO) and at harvest in tanks with *Pseudomugil signifer*.
Experimental introduction, n=24; experimental harvest, n=23.

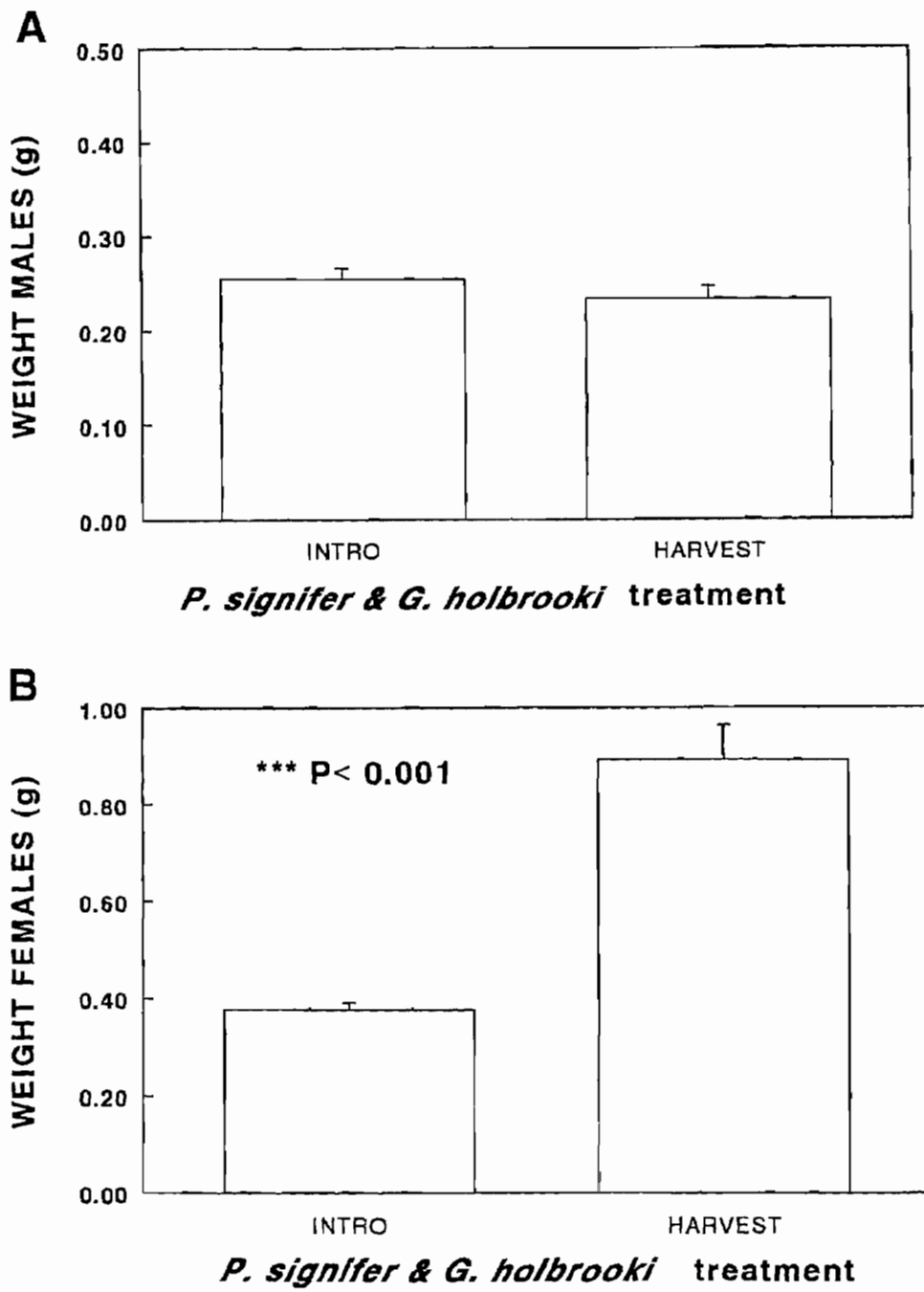


Figure 4.15 The weight of male (A) and female (B) individuals (mean ± SE) of *Gambusia holbrooki* at introduction (INTRO) and at harvest in tanks with *Pseudomugil signifer*.
Males, experimental introduction, n=12; experimental harvest, n=11.
Females, experimental introduction, n=24; experimental harvest, n=23.

Numbers of juveniles *P. signifer* and *G. holbrooki.* Fig. 4.16 shows the mean number of juvenile *P. signifer* and *G. holbrooki* produced over the duration of the experiment. In the presence of *G. holbrooki*, *P. signifer* did not spawn. In contrast, *P. signifer* produced young in the absence of *G. holbrooki*. *G. holbrooki* however, produced young in the presence of *P. signifer*. In this initial experiment the fish were not examined for fecundity.

Synthesis of results, experiment I.

Table 4.5 provides a summary of the results obtained from Experiment I on survival numbers and growth parameters of adult P. signifer and *G. holbrooki*.

4.3.1.3 Summary of findings.

The results indicate that the presence of *G. holbrooki* had substantial effects on the growth of *P. signifer* over the period of the experiment. Both male and female *P. signifer* were reduced in numbers when housed or not housed with *G. holbrooki*. For male *P. signifer* the decline in proportion of individuals by harvest was greater in the presence of *G. holbrooki*. In contrast no change in the survival of male or female *G. holbrooki* was found. Among males of *P. signifer* housed without *G. holbrooki* there was an increase in standard length and weight but no increase in total length.

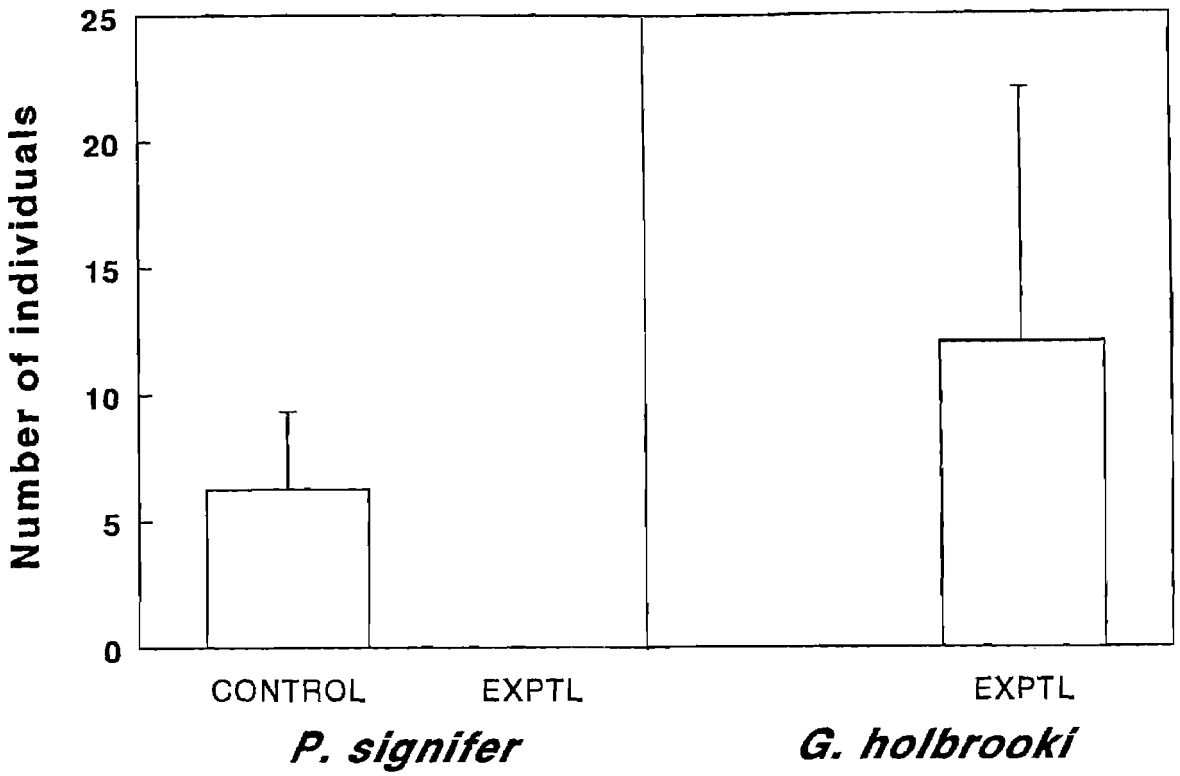


Figure 4.16 The mean number of juveniles (mean ± SE, n=4) of *Pseudomugil signifer* and *Gambusia holbrooki* at harvest in tanks with adult *P. signifer* only (CONTROL) and tanks with adult *P. signifer* and *G. holbrooki* (EXPTL). G. holbrooki produced young in the presence of *P. signifer*.

Table 4.5. Summary of analysis from Experiment I, from introduction to harvest, on the impact of *G. holbrooki* on the growth of *P. signifer* (in summary from page 121).

All survivals were analysed by a one-tailed, single sample t-test. All other parameters were examined by a 2-tailed unpaired t-test.

Parameter	Significance
SUR nos. M *P. signifer* (CON)	P= 0.049
SUR nos. M *P. signifer* (EXPT)	P= 0.0081
SUR nos. F *P. signifer* (CON).	P=0.0450
SUR nos. F *P. signifer* (EXPT).	P= 0.0177
SUR nos. M *G. holbrooki* (EXPT).	N.S.
SUR nos. F *G. holbrooki* (EXPT).	N.S.
SL M *P. signifer* (CON)	P= 0.0019
SL M *P. signifer* (EXPT)	N.S.
SL F *P. signifer* (CON)	P< 0.0001
SL F *P. signifer* (EXPT)	N.S.
SL M *G. holbrooki* (EXPT)	N.S.
SL F *G. holbrooki* (EXPT)	P<0.0001
TL M *P. signifer* (CON)	N.S.
TL M *P. signifer* (EXPT)	N.S.
TL F *P. signifer* (CON)	P<0.0001
TL F *P. signifer* (EXPT)	N.S.
TL M *G. holbrooki* (EXPT)	N.S.
TL F *G. holbrooki* (EXPT)	P<0.0001
WT M *P. signifer* (CON)	P< 0.0023
WT M *P. signifer* (EXPT)	N.S.
WT F *P. signifer* (CON)	P<0.0011
WT M *G. holbrooki* (EXPT)	N.S.
WT F *G. holbrooki* (EXPT)	P<0.0001

KEY

SUR nos., number surviving; CON, Treatment with *P. signifer* only; EXPT, Treatment with *P. signifer* and *G. holbrooki*.

M, male; F, female; N.S., not significantly different;

SL, standard length; TL, total length; WT, weight.

Females of *P. signifer* housed without *G. holbrooki* grew substantially in both length parameters and in weight. In both male and female *P. signifer* housed with *G. holbrooki* no increase in length parameters or weight was found. In males of *G. holbrooki* no difference was found in any of the measured growth parameters. In contrast, females of *G. holbrooki* increased substantially in all growth parameters measured.

4.3.2. Tank experiment II - Impact of *G. holbrooki* on the fecundity of *P. signifer*, with and without augmented food supply.

The water quality results are presented first to show homogeneity between treatments and that adequate water quality was maintained through the experiment. Details of the specific results on survival rates, and measures of growth and reproductive activity are presented on *P. signifer*, then *G. holbrooki*, summarised in Tables 4.8 and 4.9.

4.3.2.1 Water quality.

pH (Fig. 4.17A). The median pH values of the water (range at the beginning of experiment 8.0-8.9, range at harvest 9.1-10.6) were not significantly different between the four tank treatments at the introduction or at harvest. The median Ph values in all four treatments increased significantly from the beginning of the experiment in October to harvest in December. All treatments yielded the same statistics ($P = 0.0286$, Mann-Whitney test).

Temperature (Fig. 4.17B). The temperatures found in the tanks were comparable to those in the field (range at the start of the experiment was 15.0-15.3°C, range at harvest 24.5-26.5°C). The temperature range at which *P. signifer* have been found in the field is 15-28°C (Allen and Cross, 1982). There was no significant difference between the tank treatments at the introduction or at harvest. The median temperatures increased significantly in all treatments by the time of harvest ($P = 0.0286$, Mann-Whitney test) (Note: all treatments yielded the same statistics).

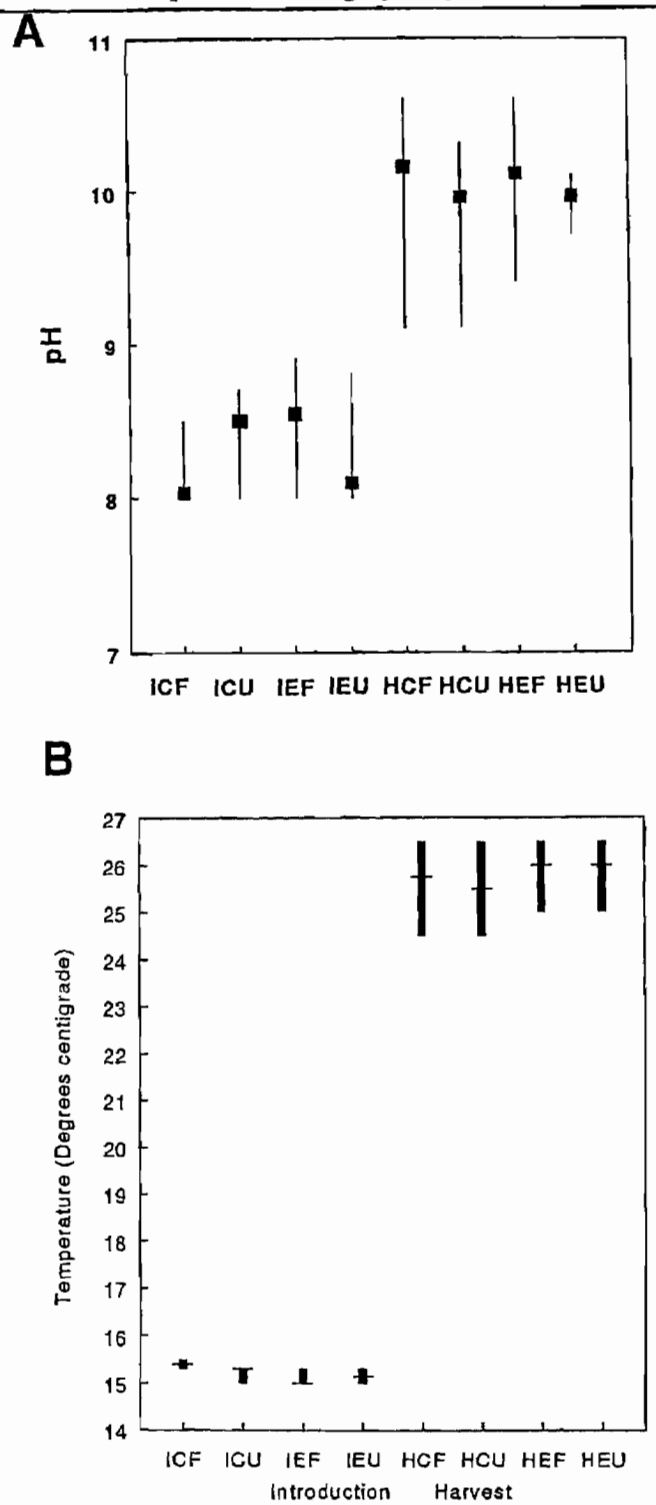


Figure 4.17 pH (A) and temperature (B) (range and medians, n=4) of tanks with *Pseudomugil signifer* only, with supplementary food and without supplementary food and tanks with *P. signifer* and *Gambusia holbrooki* with supplementary food and without supplementary food measured at introduction and harvest.

ICF - introduction control fed
ICU - introduction control unfed
IEF - introduction experimental fed
IEU - introduction experimental unfed
HCF - harvest control fed
HCU - harvest control unfed
HEF - harvest experimental fed
HEU - harvest experimental unfed

Dissolved oxygen (Fig. 4.18A). The dissolved oxygen concentration at both the introduction and harvest of this experiment fell within the acceptable range (above 3mg/l) according to Alabaster and Lloyd (1982). There was however a significant decline in the level of dissolved oxygen levels in all treatments from introduction to harvest (Control fed $P=0.0094$; Control unfed $P=0.0003$; Experimental fed $P=0.0128$ and Experimental unfed $P<0.0001$; unpaired t-test). No difference was found between the tank treatments at the introduction or at harvest.

Biological oxygen demand (BOD) (Fig. 4.18B). At the beginning of this experiment the BODs were found to be significantly different from each other (Control fed versus Experimental unfed $P<0.05$; Control unfed versus Experimental unfed $P<0.05$, one- way ANOVA with Tukey Kramer multiple comparisons test). Specifically the experimental tanks that were not given supplementary food had a slightly elevated BOD. However all the BODs at introduction were low and therefore considered of acceptable water quality and not of great concern to the overall results. At harvest the BOD values in the different tank treatments were not significantly different from one another. However there was a significant increase in the BOD levels from introduction to harvest in all treatments (Control fed $P=0.0004$; Control unfed, Experimental fed and Experimental unfed all $P<0.0001$, unpaired t-test).

Ammonia (Fig. 4.19A). The un-ionised ammonia levels were not significantly different between the tank treatments at the introduction or at harvest.

Nitrate (Fig 4.19B). The nitrate levels were low (<5 μg/l). No difference was found in the nitrate levels between control and experimental tanks (one-way ANOVA).Nitrate levels did not increase from introduction to harvest (unpaired t-test).

Hardness (Fig. 4.20). The hardness levels were not significantly different between the tank treatments at introduction or at harvest (one-way ANOVA). The hardness levels increased significantly from introduction to harvest (Control fed, $P=0.0758$; Control unfed, $P=0.0067$; Experimental fed, $P=0.0086$ and Experimental unfed, $P = 0.0540$; unpaired t-test).

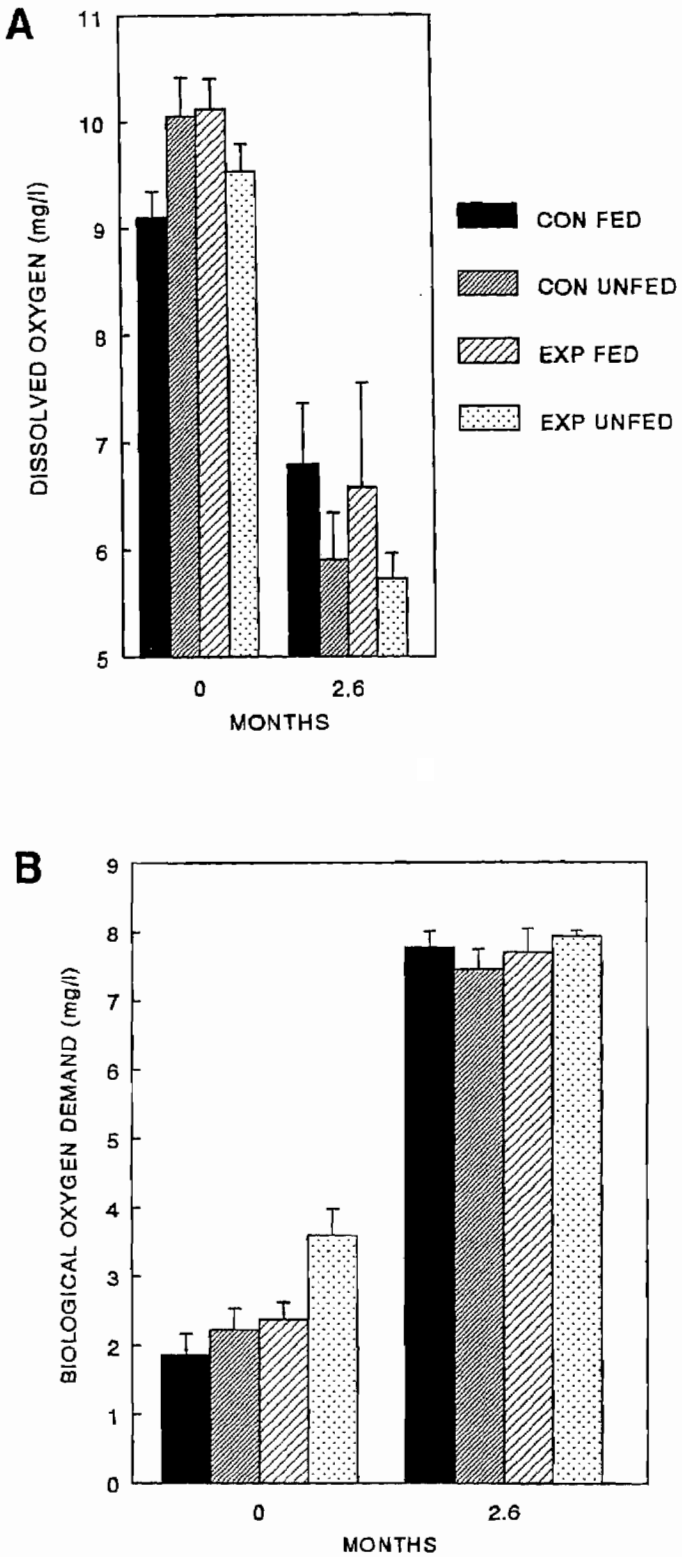


Figure 4.18 The dissolved oxygen concentrations (A) and biological oxygen demand (B) (mean ± SE, n=4) of tanks with *Pseudomugil signifer* only, with supplementary food (CON FED) and without supplementary food (CON UNFED) and tanks with *P. signifer* and *Gambusia holbrooki* with supplementary food (EXP FED) and without supplementary food (EXP UNFED) measured at introduction and harvest.

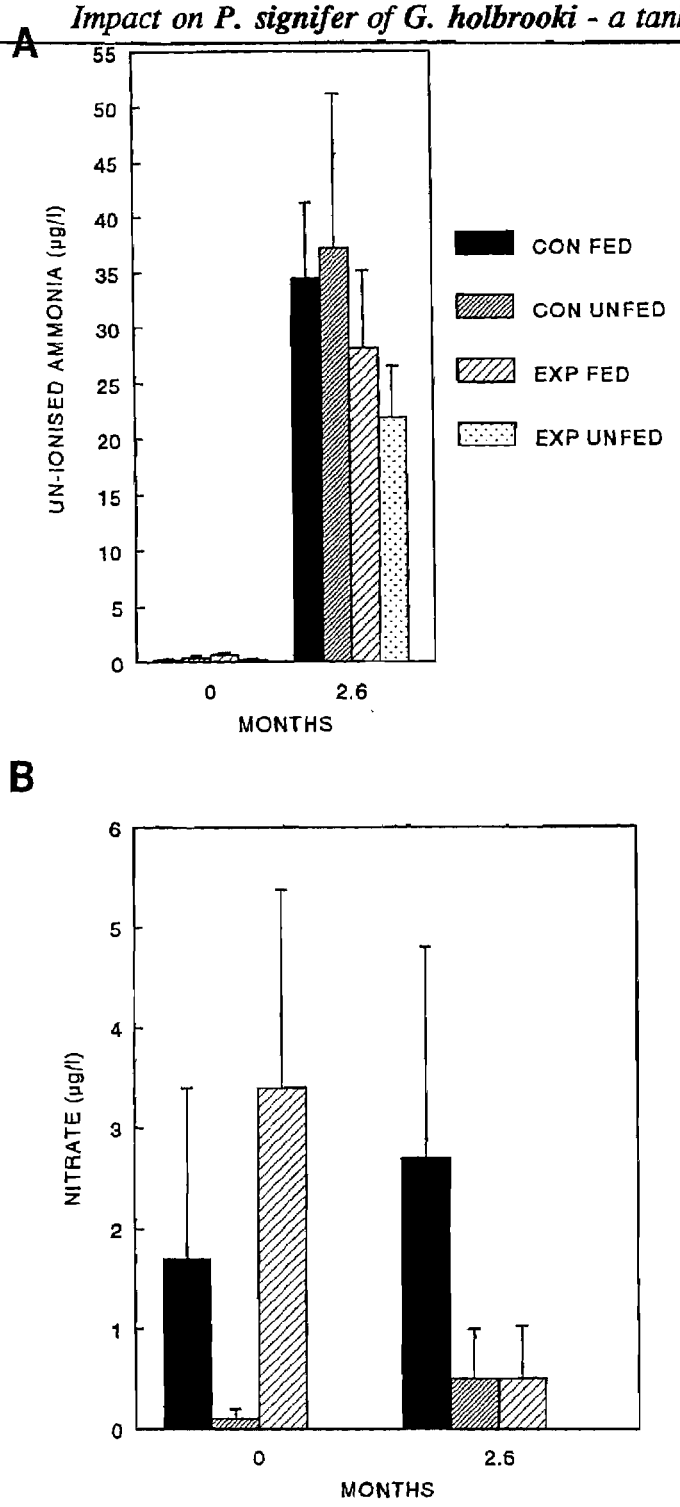


Figure 4.19 Un-ionised ammonia (A) and nitrate (B) concentrations (mean ± SE, n=4) of tanks with *Pseudomugil signifer* only, with supplementary food (CON FED) and without supplementary food (CON UNFED) and tanks with *P. signifer* and *Gambusia holbrooki* with supplementary food (EXP FED) and without supplementary food (EXP UNFED) measured at introduction and harvest.
The nitrate level for the experimental unfed treatment was undetectable at introduction and harvest.

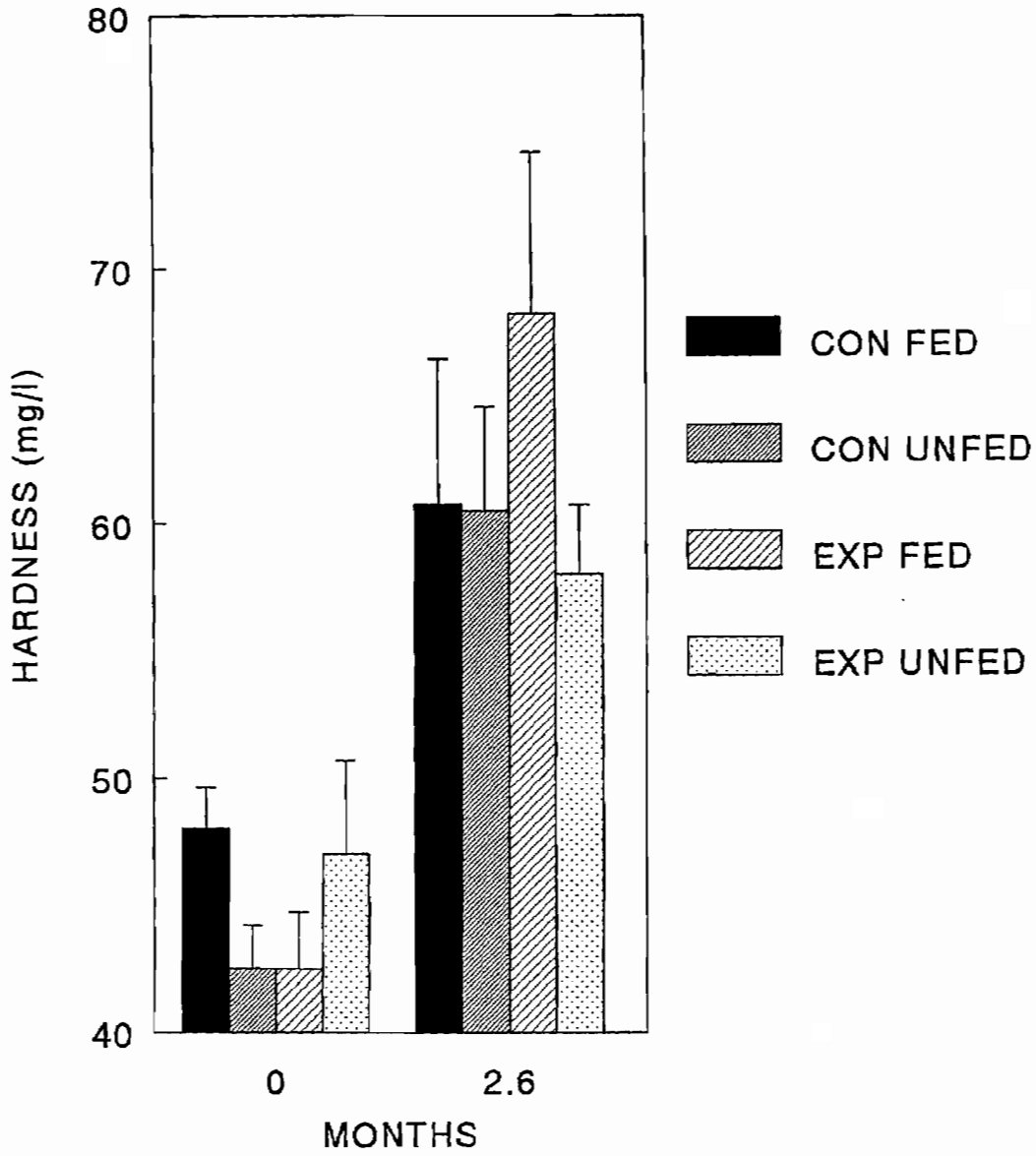


Figure 4.20 The hardness levels (mean ± SE, n=4) of tanks with *Pseudomugil signifer* only, with supplementary food (CON FED) and without supplementary food (CON UNFED) and tanks with *P. signifer* and *Gambusia holbrooki* with supplementary food (EXP FED) and without supplementary food (EXP UNFED) measured at introduction and harvest.

Invertebrates and phytoplankton.

At the beginning of the experiment mosquito larvae were present in all the tanks but none were present at harvest (Table 4.6). However there appeared to be appropriate food for the fish as invertebrates such as copepods, rotifers, midge larvae and cladocerans were present. A variety of phtytoplankton was present for invertebrates to feed on (Table 4.7).

Table 4.6 Abundance of invertebrates at introduction and at harvest in the control and experimental tanks (Mean number of organisms/litre ± standard error).

Treatment	Mosquito larvae	Copepods	*Collembola*	Midge larvae	Rotifers	Copepod naupilii	Clado-cerans
Intro Fed Control	86.2±30.2	17.1±2.0	15.0±15.0	1.4± 1.4	1,624± 133	858± 206	0
Intro Unfed Control	49.9±19.6	12.1±1.8	0	2.9± 1.7	1,264± 394	576± 129	0.7± 0.7
Intro Fed Expt	45.6±35.1	17.8±6.5	0	3.6± 1.4	1,458± 535	870± 247	3.6± 1.8
Intro Unfed Expt	13.5±7.0	12.8±4.7	8.6±5.4	1.4± 1.4	2,987± 676	635± 235	1.4± 1.4
Har Fed Control	0	0	0	0.7± 0.7	1,264± 1,188	18± 18	6.8± 3.5
Har Unfed Control	0	9.3±4.6	0	1.4± 0.8	29± 29	59± 24	32.1± 8.0
Har Fed Expt	0	4.3±4.3	0	3.6± 2.1	282± 223	117± 41	10.7± 3.2
Har Unfed Expt	0	0	0	1.4± 1.4	882± 864	59± 59	36.3± 17.3

Table 4.7 Abundance of phytoplankton at introduction and at harvest in the control and experimental tanks (Mean number of organisms/litre ± Standard Error n= 20).

Treatment	Algae 1	Algae 2	Algae 3	Algae 4	Algae 5
Intro Fed Control	412±311	4733±3110	0	1822±1611	88±88
Intro Unfed Control	135±76	694±218	0	4428±3798	0
Intro Fed Exptl	0	1399±412	0	1999±1541	47±47
Intro Unfed Exptl	176±112	1076±547	0	400±223	0
Harvest Fed Control	29±29	0	67,855±53,626	2664±1229	109,074±66,502
Harvest Unfed Control	911±459	29±29	227,380±91,846	3810±1441	9760±4616
Harvest Fed Exptl	2134±1582	0	340,746±99,724	4969±2017	19,433±11,501
Harvest Unfed Exptl	29±29	0	132,829±37,514	12,818±5491	2617±1611

Algae 1 Phylum Chlorophyta Order Chlorococcales **Crucigenia** 75μm
2 Phylum Chrysophyta Chrysomonad 100μm
3 Phylum Chlorophyta **Chlorella** 10-15μm
4 Filamentous Chlorophytes (cells 75x20μm)
5 Phylum Chlorophyta **Oocystis** 40μm

4.3.2.2 Fish parameter measurements.

P. signifer

Survival rates of fish (Fig 4.21). The numbers surviving to harvest in both sexes in all treatments (control and experimental, with and without supplementary feeding) declined significantly (Male experimental fed, P=0.0016; Female experimental fed, P=0.0402; Male control fed, P=0.0288; Female control fed, P=0.0011; Male experimental unfed, P=0.0081; Female experimental unfed, P=0.0052; Male control unfed, P=0.0031 and Female control unfed, P=0.0005; 1-tailed single sample t-test).

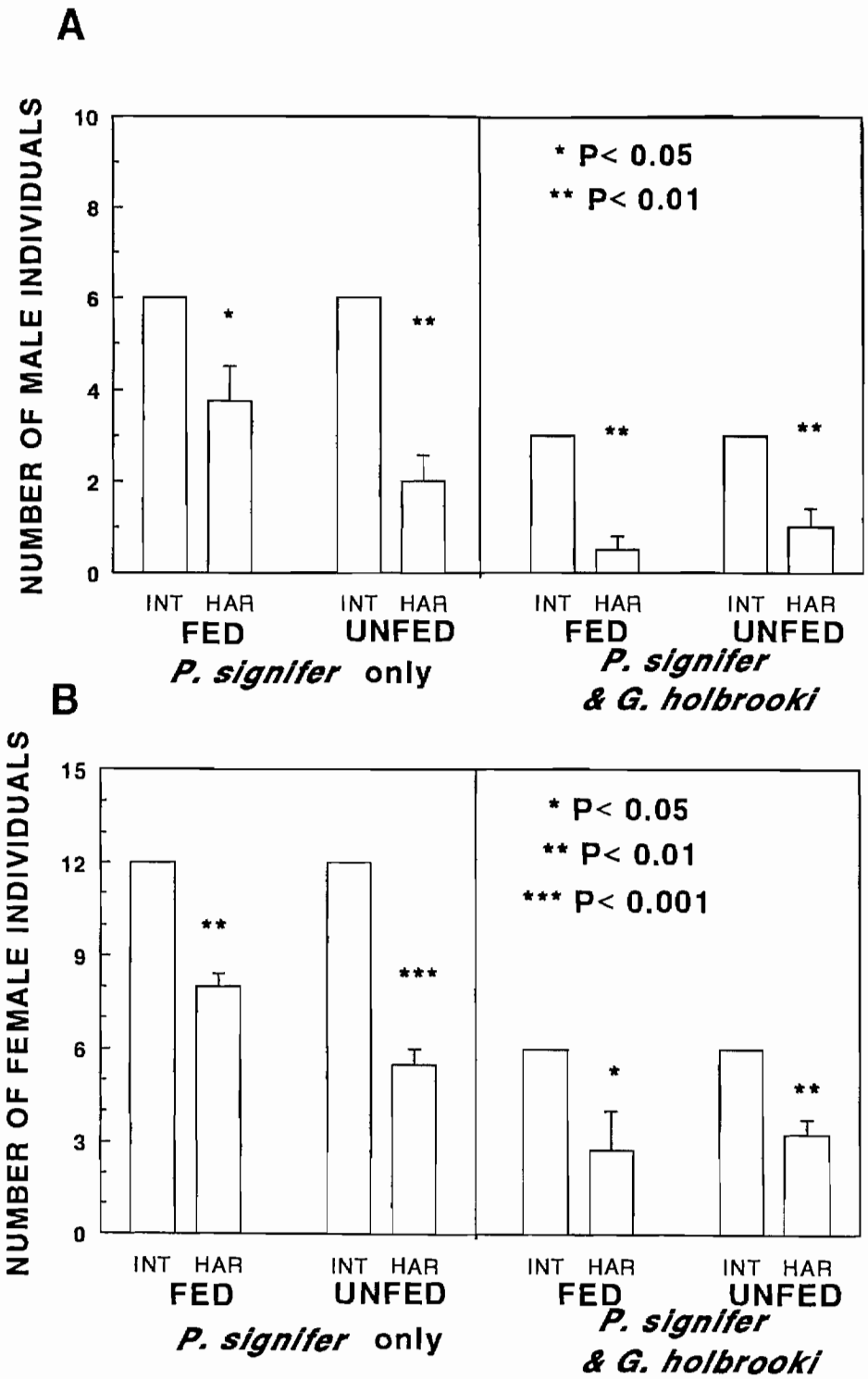


Figure 4.21 The mean number of individuals male (A) and female (B) *Pseudomugil signifer* (mean ± SE, n=4) at introduction (INT) and at harvest (HAR) in tanks with *P. signifer*, that were given supplementary food (FED) and not given supplementary food (UNFED) and tanks with *P. signifer* and *Gambusia holbrooki* that were given supplementary food and not given supplementary food.

In males no effect of feeding on survival was shown at harvest (unpaired t-test). However, in the females, feeding in the absence of *G. holbrooki* (controls) was associated with increased survival of female *P. signifer* (P=0.0082, 2-tailed t-test). This was the only occasion when any association with feeding was observed in either sex. Proportions remaining at harvest were examined (Kruskal-Wallis nonparametric ANOVA). No differences were found between groups.

Total and standard length (Fig. 4.22, 4.23). The treatment groups were homogeneous with respect to variance at the commencement of the experiment with respect to total and standard lengths (Bartlett's test for homogeneity of variances). Total length increased in the supplementary fed control males from introduction to harvest (P= 0.0418, unpaired t-test). Where it was possible to test for homogeneity of variances between tanks at harvest the samples were homogeneous. However in the males the small number of surviving fish in experimental tanks procluded examination of homogeneity of variances. There was no significant growth in total length in male fish in any other treatment. There was no significant growth in standard length in male fish in any treatment. At harvest the total and standard length of control male *P. signifer* were not significantly different from experimental *P. signifer* whether or not there had been supplementary feeding (one-way ANOVA).

In females there was growth in total length in supplementary fed and unfed controls (P= 0.0050 and 0.0001 respectively, unpaired t-test) but not in fed or unfed experimental tanks. At harvest, both total and standard lengths were greater in unfed control female *P. signifer*, compared with unfed female *P. signifer* kept with *G. holbrooki* (SL, $P<0.05$; TL, $P<0.01$; one-way ANOVA with Tukey Kramer post test). No differences were found in total or standard lengths among female *P. signifer* in supplementary fed tanks.

There was growth in standard lengths of female *P. signifer* in controls whether they were not fed supplementary food (P= 0.0001, unpaired t-test) or fed supplementary food (P= 0.0003, unpaired t-test).

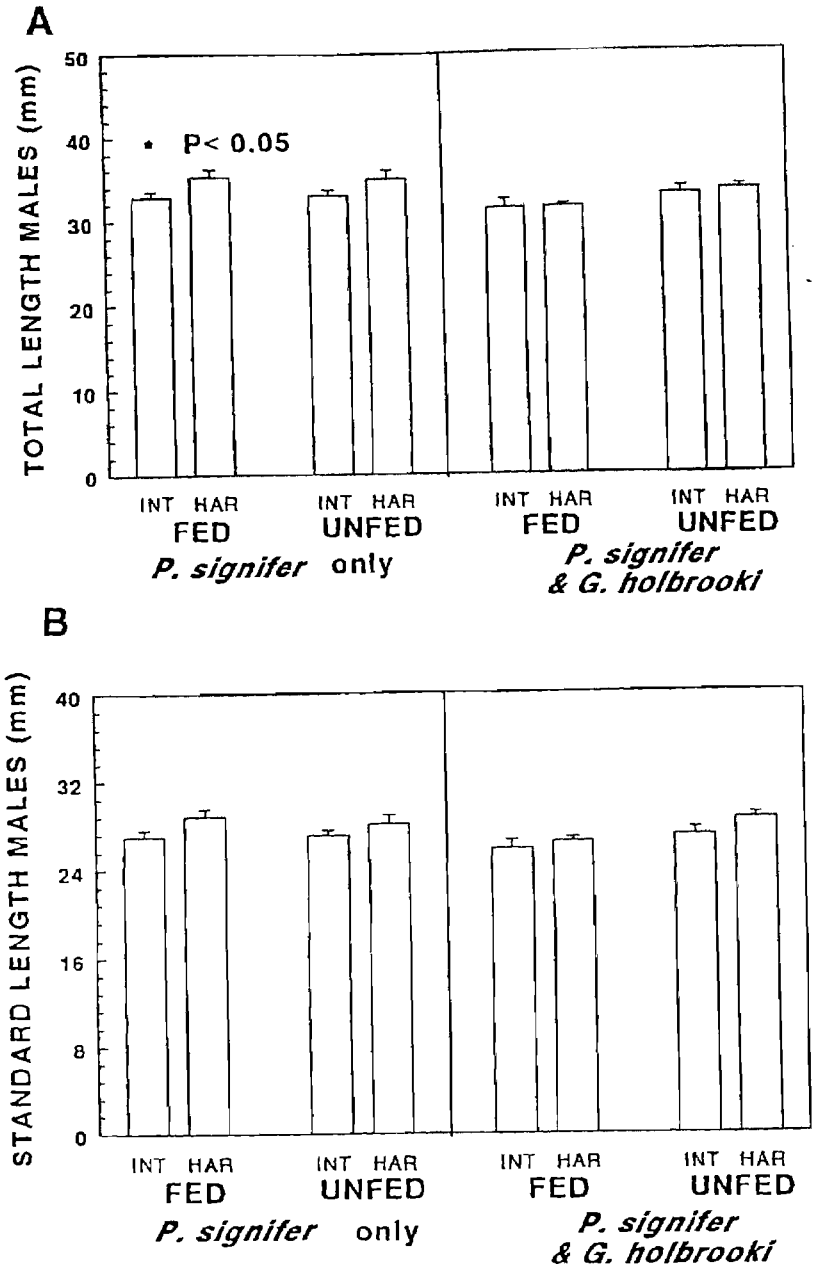


Figure 4.22 The total length (A) and standard length (B) of individuals (mean ± SE) of male *Pseudomugil signifer*, at introduction and at harvest in tanks with *P. signifer* only, that were given supplementary food (FED) and not given supplementary food (UNFED) and tanks with *P. signifer* and *Gambusia holbrooki* that were given supplementary food or not given supplementary food.
Control fed introduction, n=24; control fed harvest, n=15.
Control unfed introduction, n=24; control unfed harvest, n=8.
Experimental fed introduction, n=12; experimental fed harvest, n=2.
Experimental unfed introduction, n=12; experimental unfed harvest, n=4.

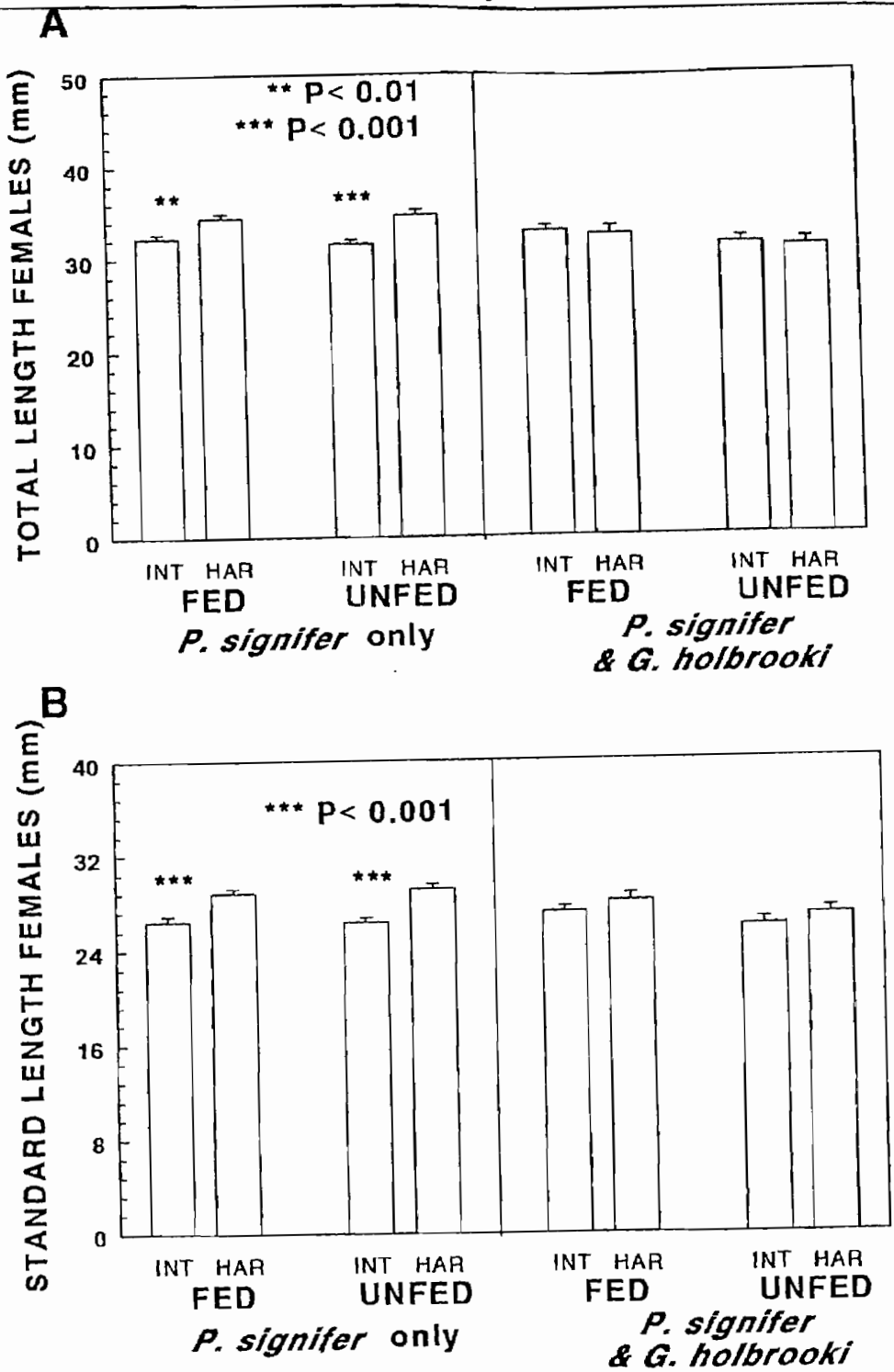


Figure 4.23 The total length (A) and standard length (B) of individuals (mean ± SE) of female *Pseudomugil signifer*, at introduction and harvest in tanks with *P. signifer* only, that were given supplementary food (FED) and not given supplementary food (UNFED) and tanks with *P. signifer* and *Gambusia holbrooki* that were given supplementary food or not given supplementary food.
Control fed introduction, n=48; control fed harvest, n=32.
Control unfed introduction, n=48; control unfed harvest, n=22.
Experimental fed introduction, n=24; experimental fed harvest, n=11.
Experimental unfed introduction, n=24; experimental unfed harvest, n=13.

Weight (Fig. 4.24). In males of *P. signifer* the weight increased from introduction to harvest only in the absence of *G. holbrooki* whether supplementary feeding was given or not (Fed, P=0.0052; Unfed, P=0.0012; unpaired t-test). In females there was an increase in weight from introduction to harvest among fish in control tanks with no supplementary feeding (P=0.0064, unpaired t-test) but no increase in weight could be found among control *P. signifer* that were given supplementary feeding (unpaired t-test).

No weight increase in *P. signifer* of either sex was found in the presence of *G. holbrooki* whether additional food was given or not.

At harvest, fish from the control treatment were heavier than those from experimental tanks whether they had additional food or not ($P<0.01$ in both cases,one-way ANOVA with Tukey-Kramer multiple comparisons test). No specific effect of additional feeding on weight could be shown in either sex.

Fecundity (Fig 4.25, 4.26A).

Fecundity in *P. signifer* was greatly reduced in the presence of *G. holbrooki* whether the fish were given supplementary food or not ($P<0.001$ fed, $P<0.01$ unfed, Kruskal-Wallis nonparametric ANOVA with Dunn's post-test). No effect with additional feeding could be shown whether or not *G. holbrooki* were present. In addition, fecundity was plotted against body weight at harvest in each treatment group (Fig 4.27). A correlation between fecundity and body weight was not present in any group except the control unfed group ($y = -21.262+86.281x$, $r^2 = 0.422$).

Ovarian weight (Fig. 4.26B). Median ovarian weight was greatly reduced in *P. signifer* in the presence of *G. holbrooki* whether the fish were given supplementary food or not ($P<0.001$ fed, $P<0.01$ unfed, Kruskal-Wallis nonparametric ANOVA with Dunn's post tests). No effect with additional feeding could be shown whether or not *G. holbrooki* were present. As with fecundity, ovarian weight was plotted against body weight at harvest in each treatment group (Fig 4.28). Again, no correlation between fecundity and body weight was present in any group except the control unfed group ($y = -0.031+0.168x$, $r^2 = 0.544$).

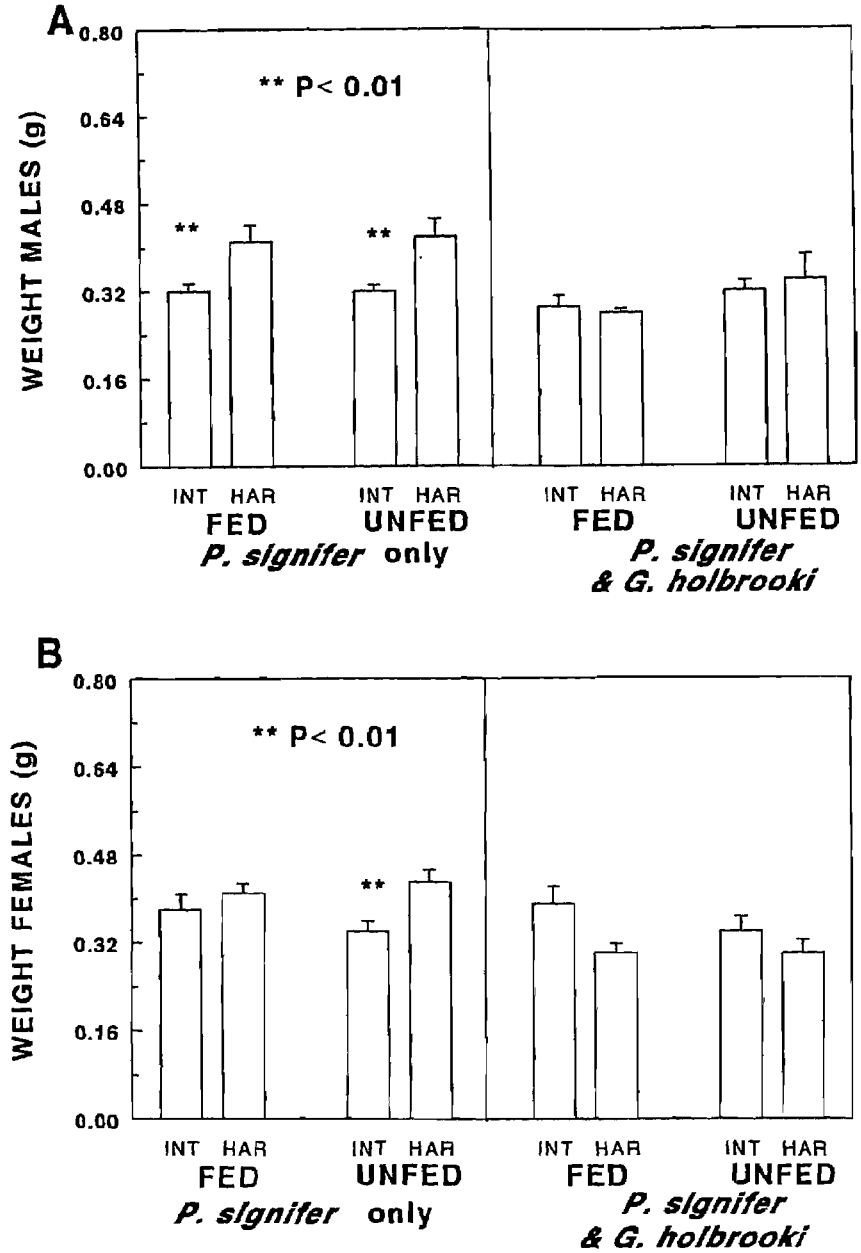


Figure 4.24 The weight of male (A) and female (B) individuals (mean ± SE) of *Pseudomugil signifer*, at introduction (INT) and harvest (HAR) in tanks with *P. signifer* only, that were given supplementary food (FED) and not given supplementary food (UNFED) and tanks with *P. signifer* and *Gambusia holbrooki* that were given supplementary food or not given supplementary food.
Male: control fed introduction, n=24; control fed harvest, n=15.
Control unfed introduction, n=24; control unfed harvest, n=8.
Experimental fed introduction, n=12; experimental fed harvest, n=2.
Experimental unfed introduction, n=12; experimental unfed harvest, n=4.
Female: control fed introduction, n=48; control fed harvest, n=32.
Control unfed introduction, n=48; control unfed harvest, n=22.
Experimental fed introduction, n=24; experimental fed harvest, n=11.
Experimental unfed introduction, n=24; experimental unfed harvest, n=13.

A

B

Figure 4.25 The ovary of *Pseudomugil signifer* at harvest. (A) Control, not housed with *Gambusia holbrooki*. Note many large eggs. (B) Experimental, housed with *G. holbrooki*. Note the small size of ovary and absence of large eggs.

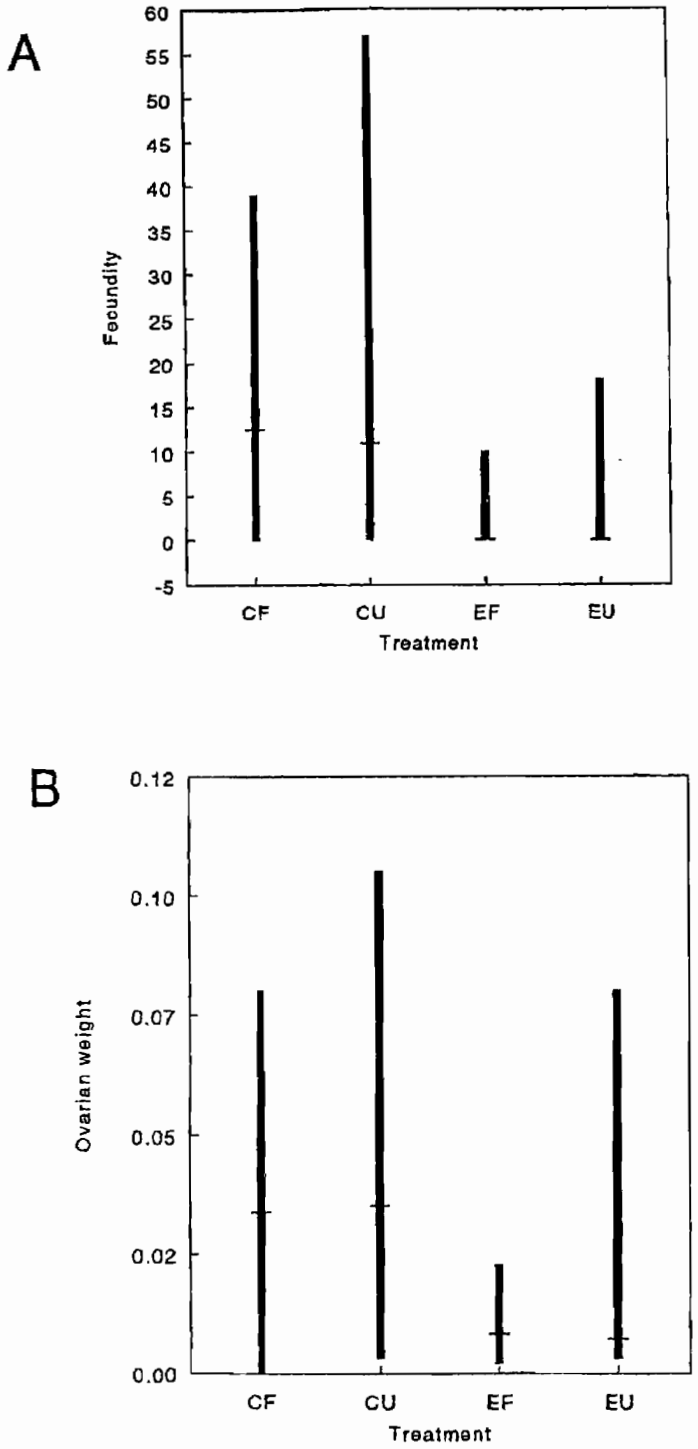


Figure 4.26 The fecundity (A) and ovarian weight (B) (median and range) of female *Pseudomugil signifer* at harvest in tanks with *P. signifer* only, that were given supplementary food (FED) and not given supplementary food (UNFED) and tanks with *P. signifer* and *Gambusia holbrooki* that were given supplementary food or not given supplementary.
Control fed harvest, n=32.
Control unfed harvest, n=22.
Experimental fed harvest, n=11.
Experimental unfed harvest, n=13.

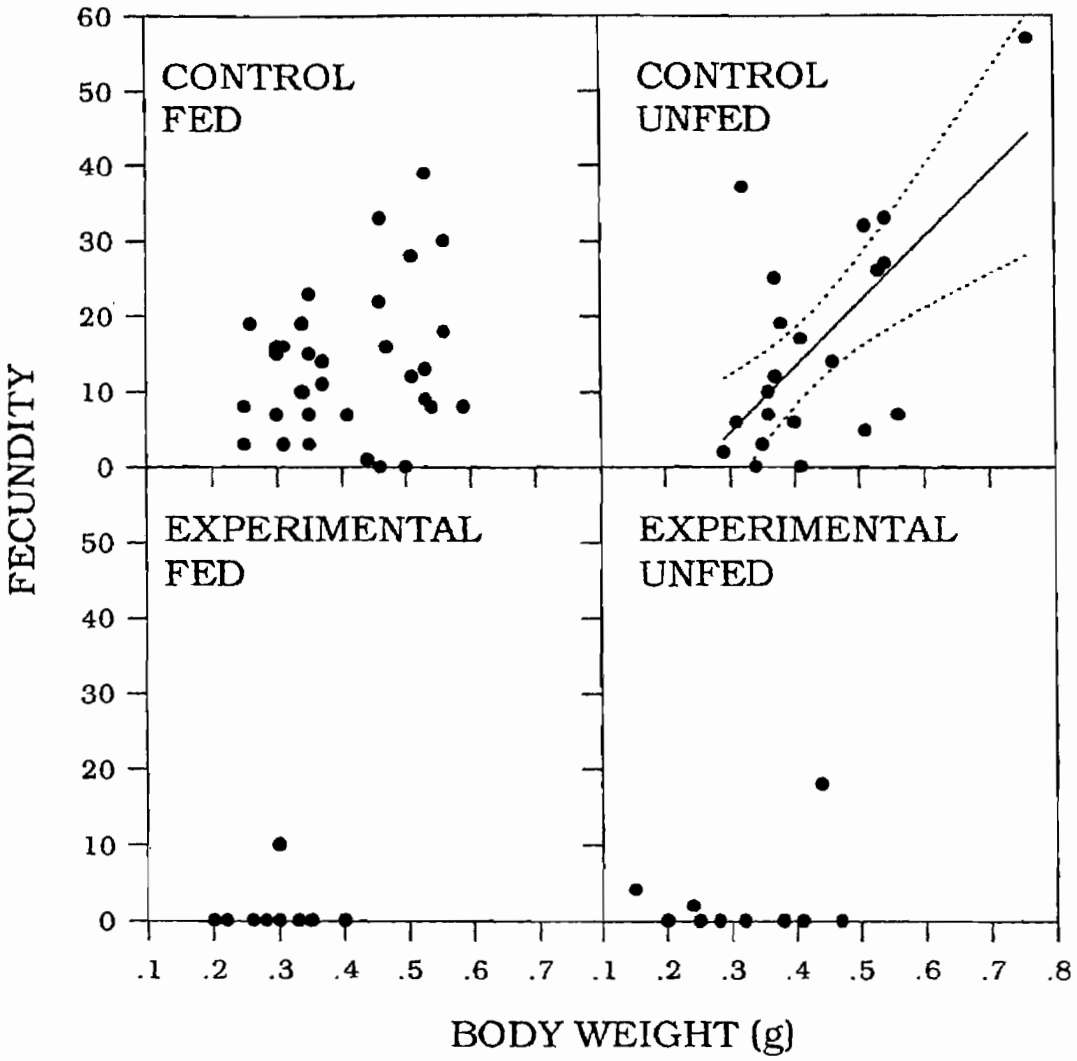


Figure 4.27. Fecundity plotted against body weight at harvest in all four treatment groups. There were no significant correlations between fecundity and body weight in any of the groups except for the control unfed group. Scales on all graphs are equal to permit comparison.

A: Control fed harvest, n=32.

B: Control unfed harvest, n=22.

Significant positive correlation between fecundity and body weight (P=0.0011).

C: Experimental fed harvest, n=11.

D: Experimental unfed harvest, n=13.

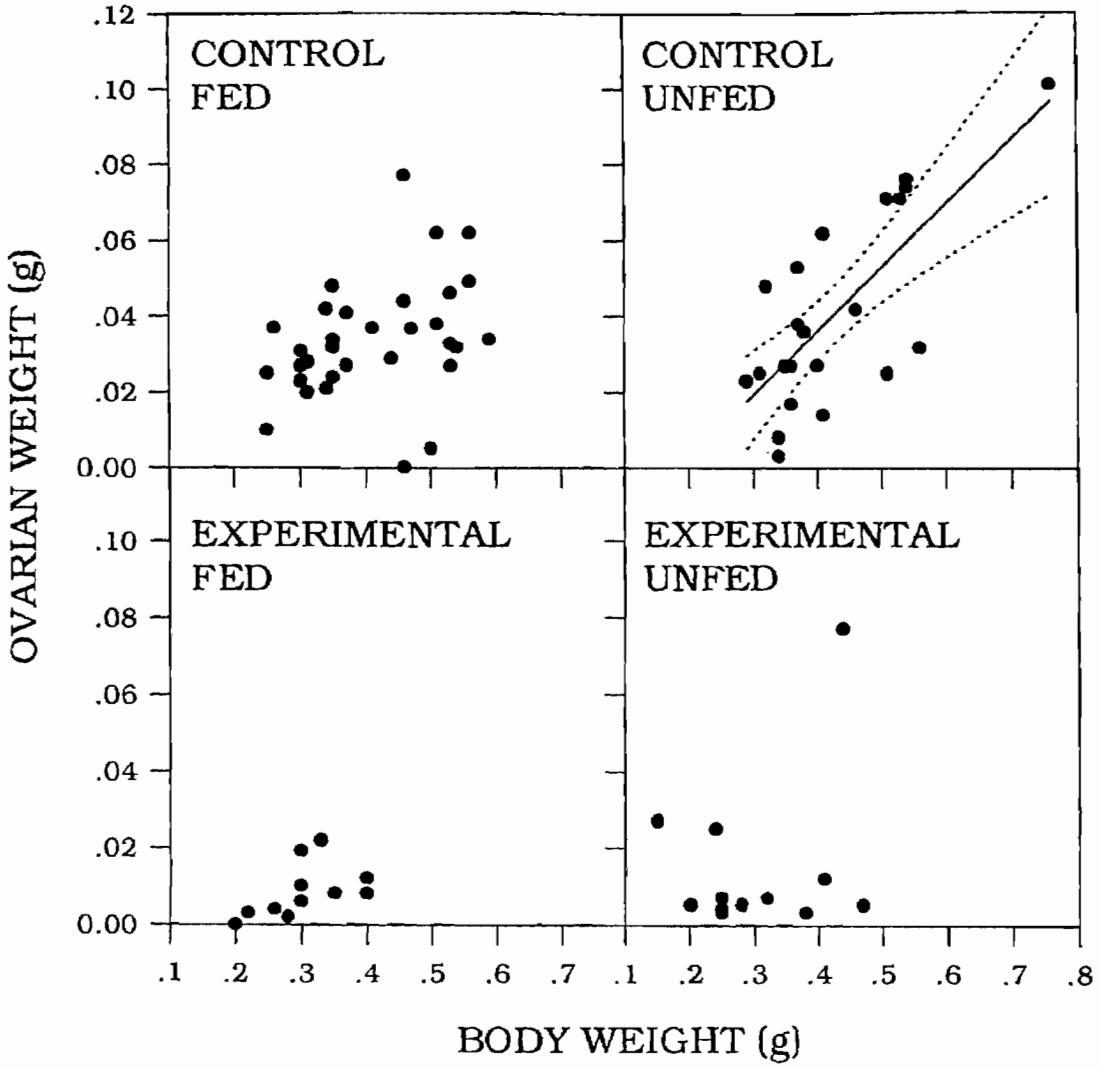


Figure 4.28. Ovarian weight plotted against body weight at harvest in all four treatment groups. There were no significant correlations between ovarian weight and body weight in any of the groups except for the control unfed group. Scales on all graphs are equal to permit comparison.

A: Control fed harvest, n=32.

B: Control unfed harvest, n=22.
Significant positive correlation between fecundity and body weight ($P < 0.0001$).

C: Experimental fed harvest, n=11.

D: Experimental unfed harvest, n=13.

Testis weight (Fig. 4.29). No difference in testis weight was found between controls whether they were or were not given supplementary feeding (one-way ANOVA). No difference was found in testis weight between *P. signifer* in experimental tanks whether given additional food or not. There was no difference between the testis weight of *P. signifer* control and experimental tanks.

Gonadosomatic index (GSI) (Fig. 4.30).

For male *P. signifer* in control tanks there was no difference in the GSI between those that were given supplementary feeding and those that were not. In addition there was no difference between the *P. signifer* in experimental tanks that were given additional food and those that were not. There was no difference in the GSI between additionally fed males of *P. signifer* from control or experimental treatments. In addition there was no significant difference in the GSI between unfed males of *P. signifer* from control or experimental treatments (one-way ANOVA).

Likewise in females of *P. signifer* no feeding effect was found in the GSI. However females of *P. signifer* from the control treatment that were given additional food had a significantly larger GSI than *P. signifer* from the experimental treatment that had been given supplementary feeding ($P<0.001$, Kruskal-Wallis nonparametric ANOVA with Dunn's post test). Females of *P. signifer* from the control treatment with no supplementary feeding had a significantly larger GSI than *P. signifer* from the experimental treatment that had not been given additional food ($P<0.05$, Kruskal-Wallis nonparametric ANOVA with Dunn's post test).

G. holbrooki.

Survival rates of fish (Fig. 4.31). There was no difference in survival in either sex, from introduction to harvest, whether or not additional food was provided (unpaired t-test). At harvest no difference was found in numbers of *G. holbrooki* in both sexes under unfed and fed regimes (unpaired t-test). Proportions remaining at harvest were examined (Kruskal-Wallis nonparametric ANOVA). No differences were found between groups.

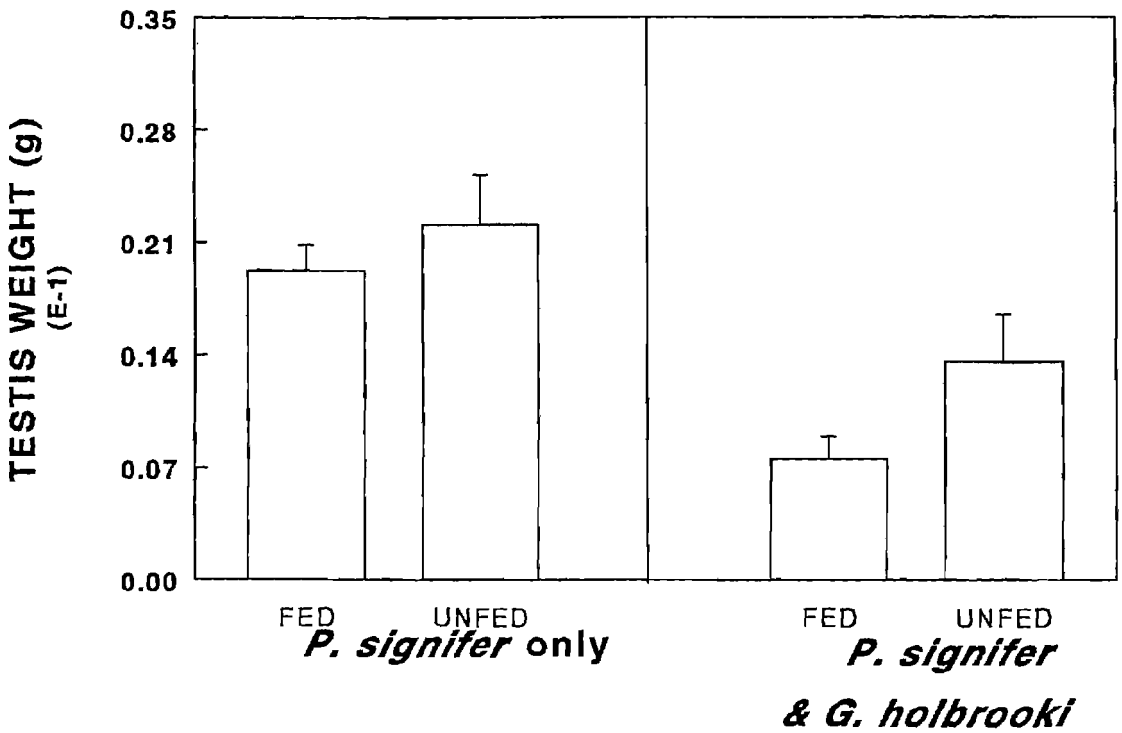


Figure 4.29 The mean testis weight (mean ± SE) of male *Pseudomugil signifer* at harvest, in tanks with *P. signifer* only, that were given supplementary food (FED) and not given supplementary food (UNFED) and tanks with *P. signifer* and *Gambusia holbrooki* that were given supplementary food or not given supplementary food.
Control fed harvest, n=15.
Control unfed harvest, n=8.
Experimental fed harvest, n=2.
Experimental unfed harvest, n=4.

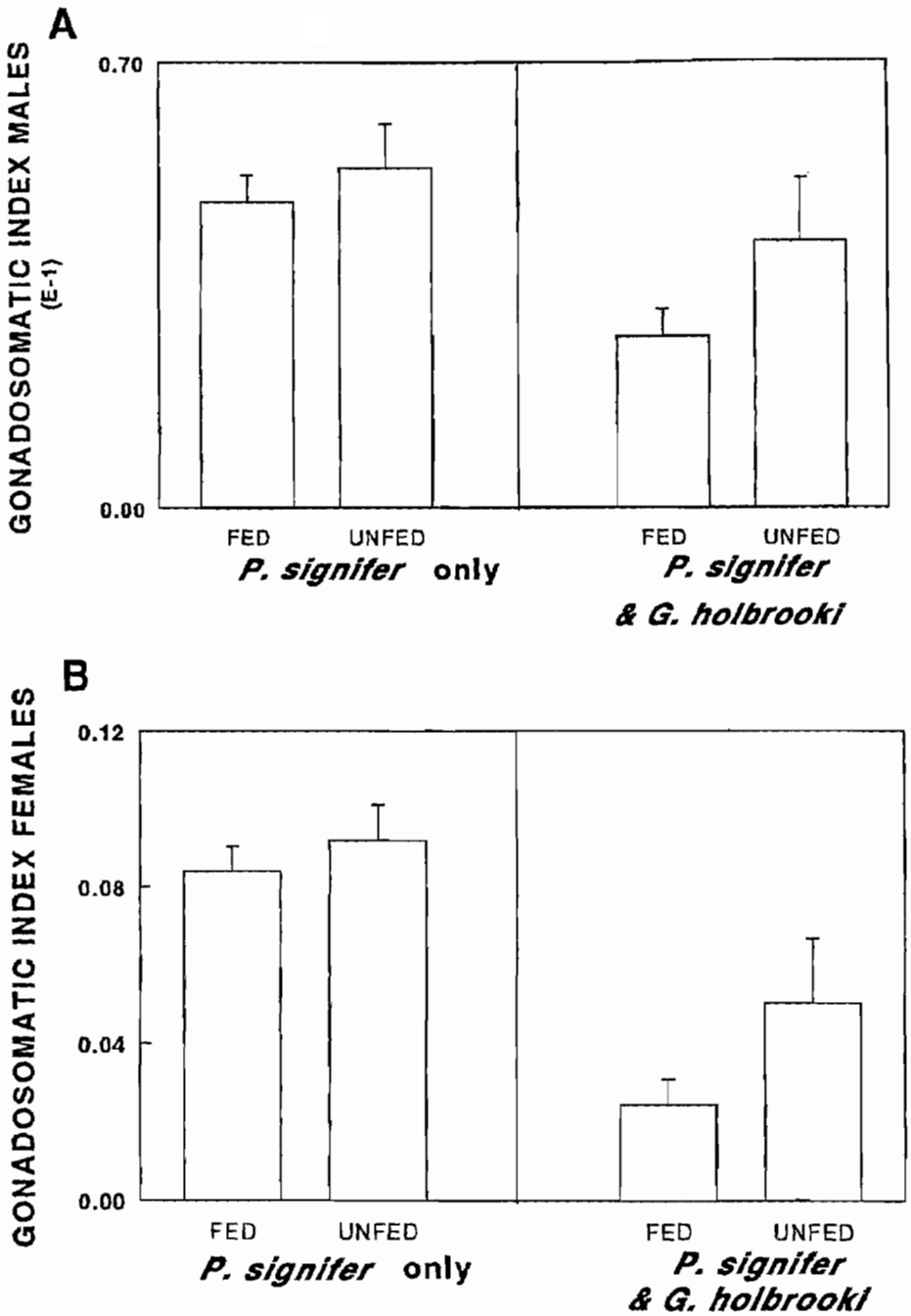


Figure 4.30 The mean gonadosomatic index (GSI) of male (A) and female (B) individuals (mean ± SE) of *Pseudomugil signifer* at harvest in tanks with *P. signifer* only, that were given supplementary food (FED) and not given supplementary food (UNFED) and tanks with *P. signifer* and *Gambusia holbrooki* that were given supplementary food or not given supplementary food.

Male: Control fed harvest, n=15. **Female**: Control fed harvest, n=32.
Control unfed harvest, n=8. Control unfed harvest, n=22.
Experimental fed harvest, n=2. Experimental fed harvest, n=11.
Experimental unfed harvest, n=4. Experimental unfed harvest, n=13.

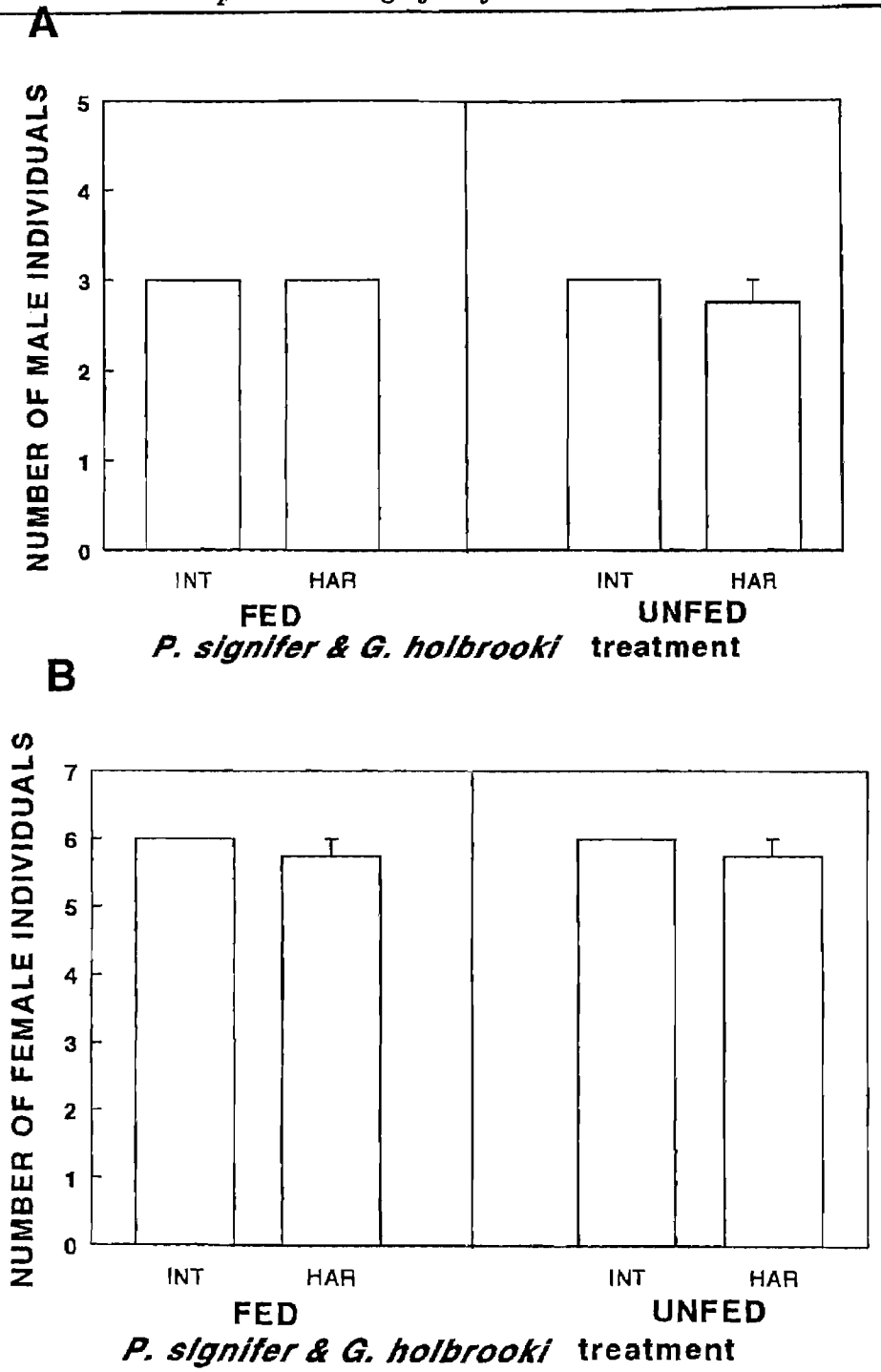


Figure 4.31 The number of individual (mean ± SE, n=4) male (A) and female (B) *Gambusia holbrooki* at introduction (INT) and at harvest (HAR) in tanks with *Pseudomugil signifer* that were given supplementary food (FED) or not given supplementary food (UNFED).

Total and standard length (Fig 4.32, 4.33). The distributions of total and standard lengths of *G. holbrooki* in the two treatments were not homogeneous at the commencement of the experiment (F statistic). The variances appeared to differ slightly. However the distributions of total and standard lengths of *G. holbrooki* at harvest in the two treatments were homogeneous (F statistic). There was growth from introduction to harvest in standard and total lengths in males when given supplementary feeding ($P=0.0065$ and 0.0115 respectively, unpaired t-test) but no significant difference in these parameters when not given additional food. No difference was found at harvest, in either total or standard lengths in males, between those that were provided with supplementary food and those that were not. Female fish grew in standard and total lengths from introduction to harvest whether provided with additional food ($P<0.0001$ both parameters, unpaired t-test) or not (SL, $P=0.0256$; TL, $P=0.0072$; unpaired t-test). No difference was found at harvest, in either total or standard lengths in females, between those that were provided with supplementary food and those that were not.

Weight (Fig. 4.34). The weights of male *G. holbrooki* declined over the experiment (supplementary fed $P=0.0022$, no supplementary food $P=0.0097$, unpaired t-test).

There was an increase in weight from introduction to harvest in both fed and unfed female *G. holbrooki* ($P<0.0001$, unpaired t-test both treatments).

No feeding effect was observed in either sex at harvest.

Fecundity (Fig. 4.35). Median fecundity was higher in *G. holbrooki* that were given supplementary feeding compared with those that were not ($P=0.0087$, Mann-Whitney test).

Gonad weight (Fig. 4.36). Median ovarian weight was larger in *G. holbrooki* housed in tanks that were given additional food ($P=0.009$, Mann-Whitney test). However, no differences were found in mean testis weights of *G. holbrooki* between the two tank treatments (additional food and no additional food; unpaired t-test).

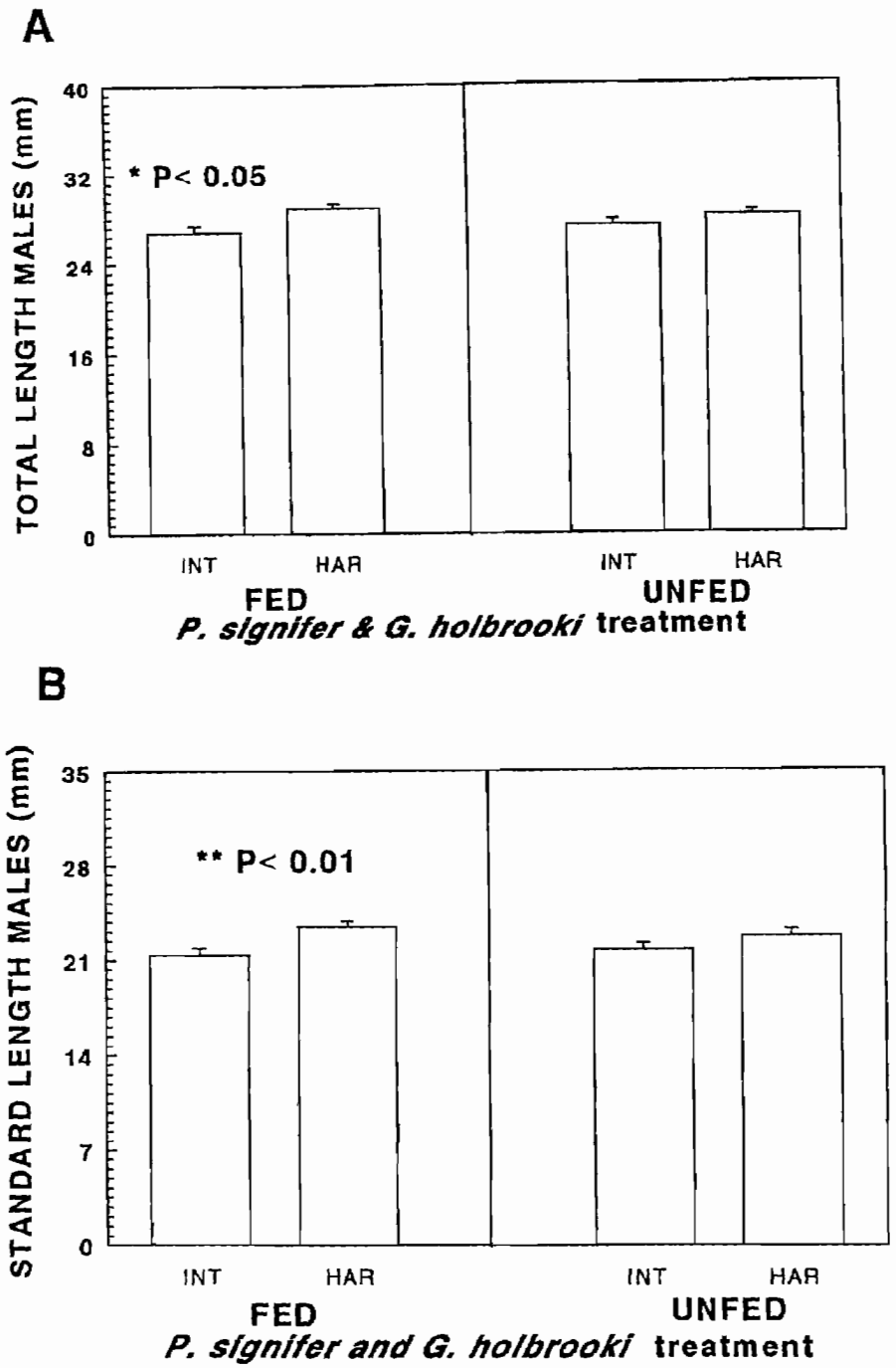


Figure 4.32 The total length (A) and standard length (B) of individuals (mean ± SE) of male *Gambusia holbrooki* at introduction (INT) and at harvest (HAR) in tanks with *Pseudomugil signifer* that were given supplementary food (FED) or not given supplementary food (UNFED).
Experimental fed introduction, n=12; experimental fed harvest, n=12.
Experimental unfed introduction, n=12; experimental unfed harvest, n=11.

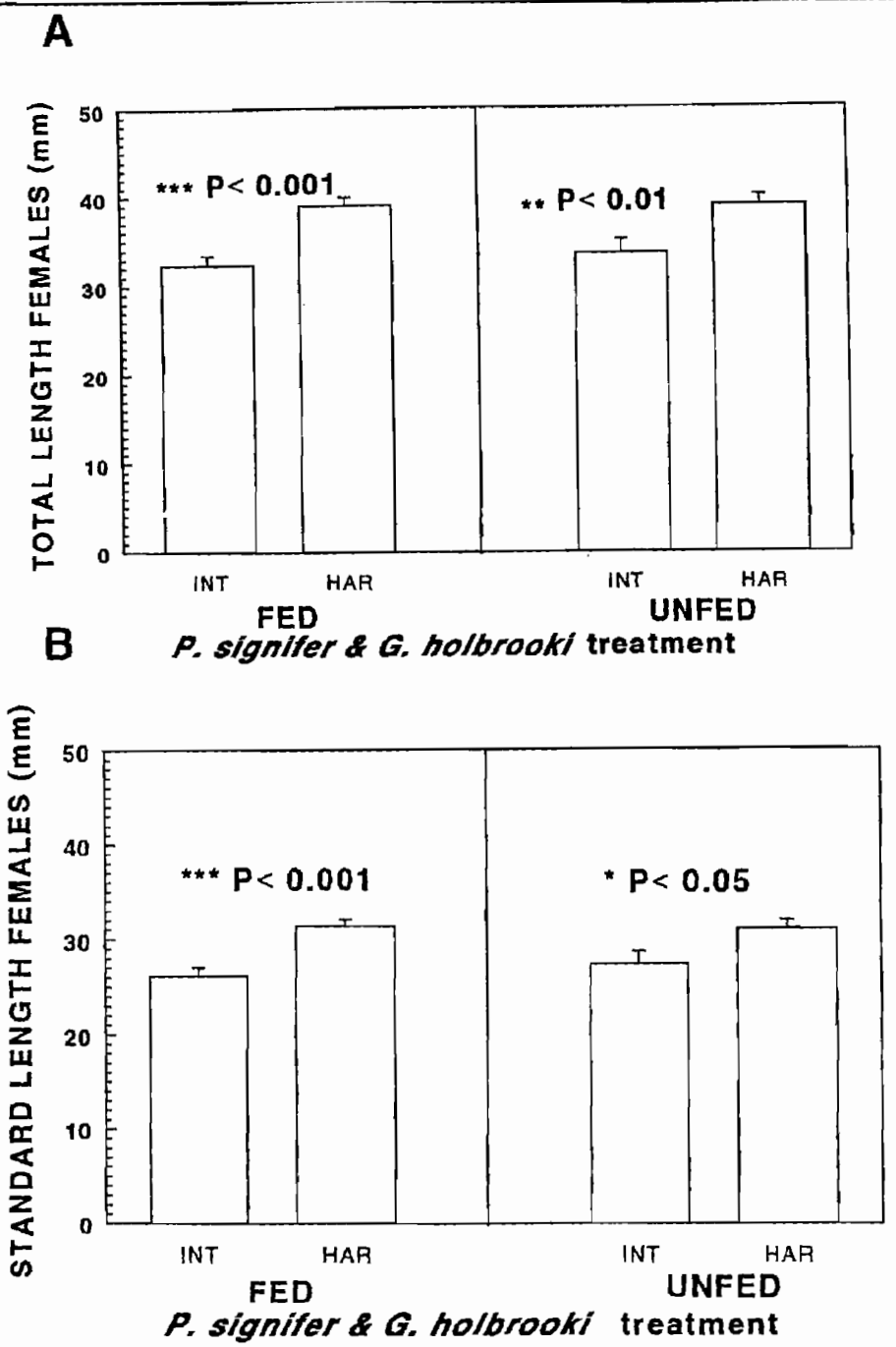


Figure 4.33 The total length (A) and standard length (B) of individuals (mean ± SE) of female *Gambusia holbrooki* at introduction (INT) and at harvest (HAR) in tanks with *Pseudomugil signifer* that were given supplementary food (FED) or not given supplementary food (UNFED).
Experimental fed introduction, n=24; experimental fed harvest, n=23.
Experimental unfed introduction, n=24; experimental unfed harvest, n=23.

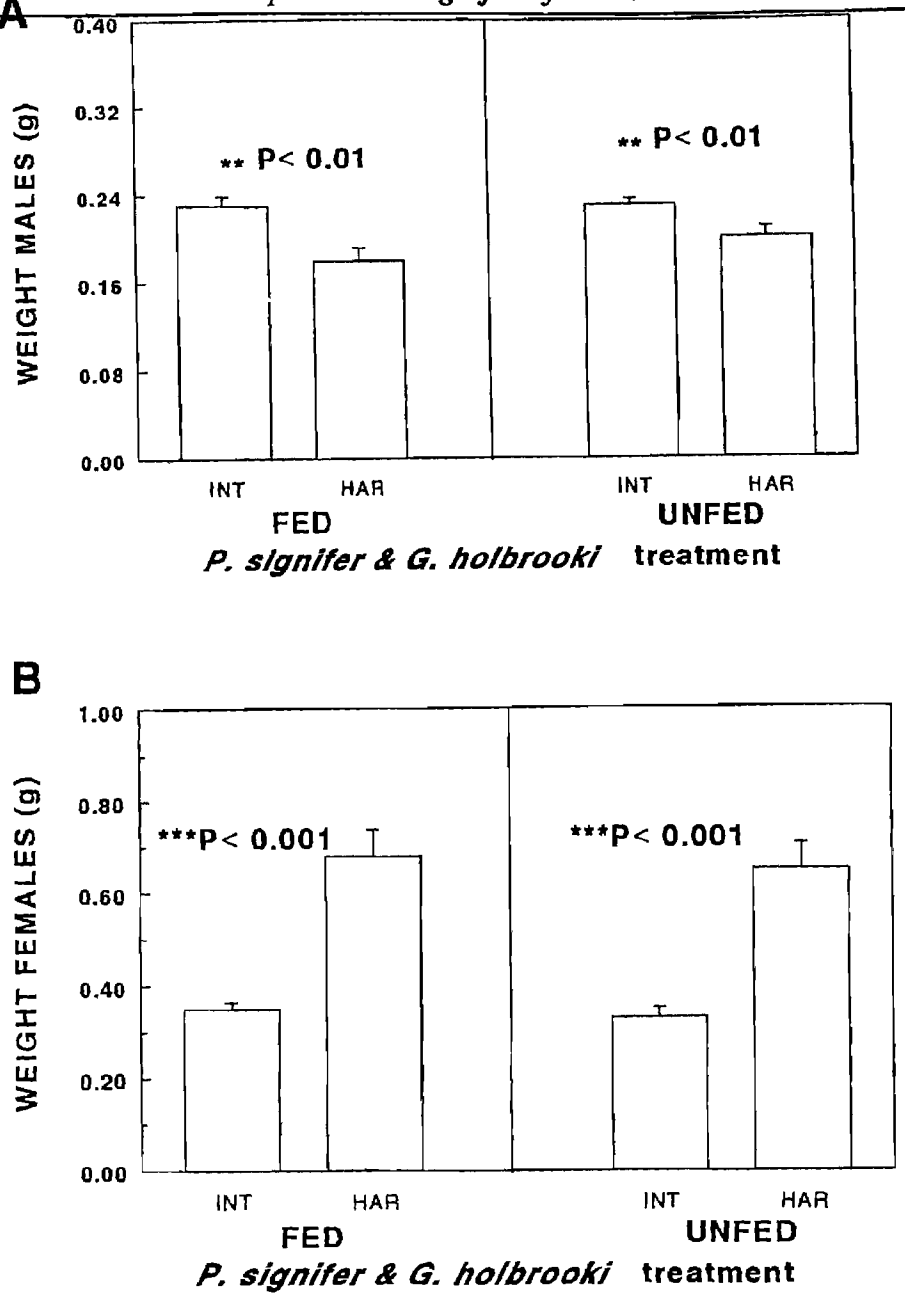


Figure 4.34 The weight of male (A) and female (B) individuals (mean ± SE) of *Gambusia holbrooki* at introduction (INT) and at harvest (HAR) in tanks with *Pseudomugil signifer* that were given supplementary food (FED) or not given supplementary food (UNFED).
Male: Experimental fed introduction, n=12; experimental fed harvest, n=12.
Experimental unfed introduction, n=12; experimental unfed harvest, n=11.
Female Experimental fed introduction, n=24; experimental fed harvest, n=23.
Experimental unfed introduction, n=24; experimental unfed harvest, n=23.

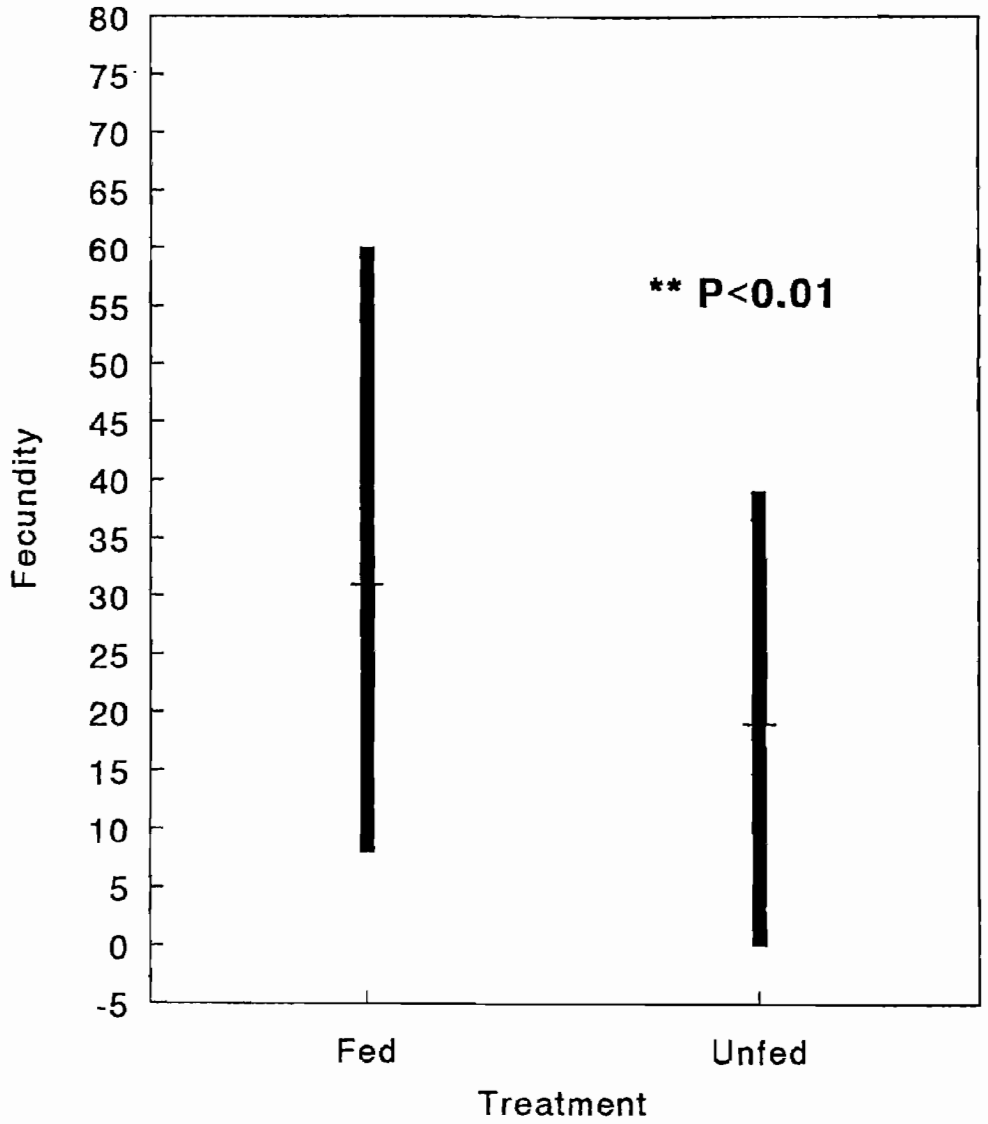


Figure 4.35 The fecundity of *Gambusia holbrooki* (median and range) at harvest in tanks with *Pseudomugil signifer* that were given supplementary food (Fed) or not given supplementary food (Unfed).
Experimental fed harvest, n=23.
Experimental unfed harvest, n=23.

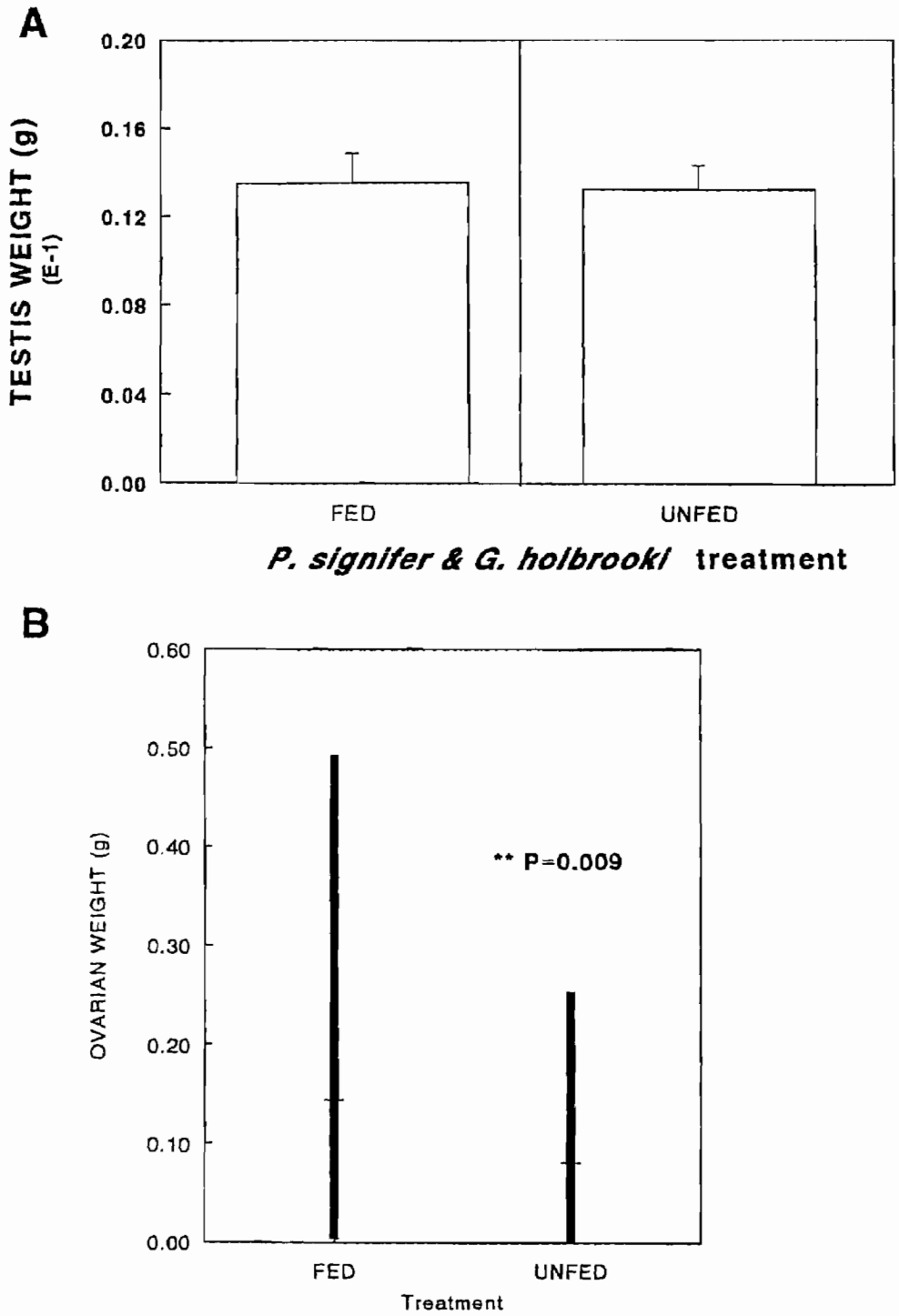


Figure 4.36 The weight of male testis (A) (mean ± SE) and female ovaries (B) (median and range) of *Gambusia holbrooki* at harvest (HAR) in tanks with *Pseudomugil signifer* that were given supplementary food (FED) or not given supplementary food (UNFED).
Male: experimental fed harvest, n=12.
Experimental unfed harvest, n=11.
Female: Experimental fed harvest, n=23.
Experimental unfed harvest, n=23.

Gonadosomatic index (GSI) (Fig. 4.37). The mean GSI in male *G. holbrooki* was not different between the two feeding treatments (unpaired t-test). The females, however, had significantly larger GSIs when housed in tanks that were given additional food ($P = 0.0007$, unpaired t-test).

A summary of the main findings on survival and growth parameters is shown in Table 4.8. A summary of the main findings on reproductive characteristics at harvest is shown in Table 4.9.

4.3.2.3 Synthesis of findings.

The results from this experiment indicated that the presence of *G. holbrooki* had dramatic effects on the growth and reproductive capacity of *P. signifer*. Growth in *P. signifer* only occurred in the absence of *G. holbrooki*, whether or not supplementary food was provided. Fecundity and ovarian weight in *P. signifer* was greatly reduced in the presence of *G. holbrooki* whether the test tanks were given supplementary food or not. With the exception of survival numbers of control female *P. signifer,* no association with supplementary feeding was observed in either sex, including the data on total and standard length and weight.

Juvenile *P. signifer* and *G. holbrooki* (Fig. 4.38). No juveniles of *P. signifer* were found at harvest. This was not unexpected as this experiment was harvested in December. Given that the fish would have required time to become accustomed to their new environment and that the eggs take approximately three weeks to hatch it was predicted that no juvenile *P. signifer* would be present at harvest. However several eggs were observed on the *Taxiphyllum barbieri* in control tanks only (in the absence of *G. holbrooki*). No difference was found in the number of juveniles of *G. holbrooki* between the tanks that had supplementary feeding and those that did not.

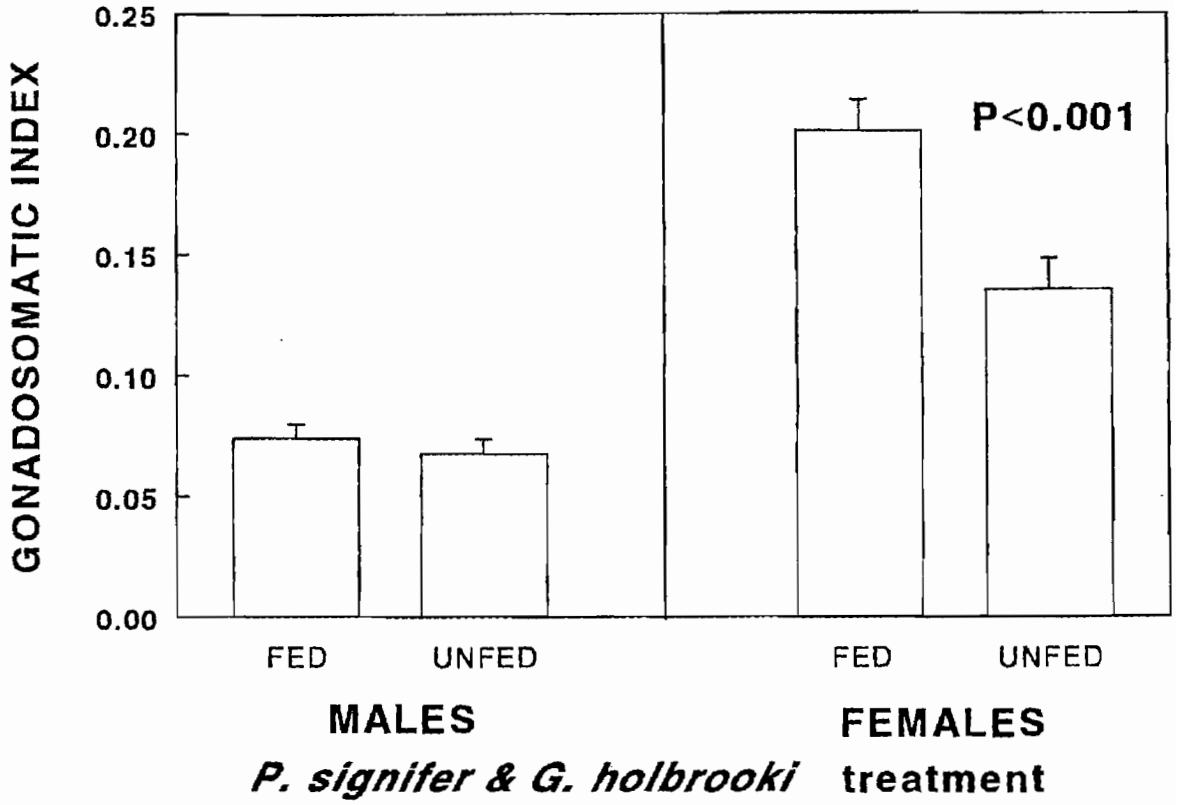


Figure 4.37 The gonadosomatic index (mean ± SE) in male and female *Gambusia holbrooki* at harvest in tanks with *Pseudomugil signifer* that were given supplementary food (FED) or not given supplementary food (UNFED).
Male: experimental fed harvest, n=12.
Experimental unfed harvest, n=11.
Female: Experimental fed harvest, n=23.
Experimental unfed harvest, n=23.

Table 4.8 A summary of changes in growth parameters (from page 141) from introduction to harvest. All survivals were analysed by a one-tailed, one sample t-test. All other parameters were examined by a two-tailed unpaired t-test.

Parameter	Significance
Sur M *P. signifer* (CON) Fed	NS
Sur M *P. signifer* (CON) Unfed	P<0.0031
Sur M *P. signifer* (EXPT) Fed	P<0.0016
Sur M *P. signifer* (EXPT) Unfed	P<0.0081
Sur F *P. signifer* (CON) Fed	P<0.0011
Sur F *P. signifer* (CON) Unfed	P<0.0005
Sur F *P. signifer* (EXPT) Fed	P<0.0402
Sur F *P. signifer* (EXPT) Unfed	P< 0.0052
Sur M *G. holbrooki* (EXPT) Fed	NS
Sur M *G. holbrooki* (EXPT) Unfed	NS
Sur F *G. holbrooki* (EXPT) Fed	NS
Sur F *G. holbrooki* (EXPT) Unfed	NS
SL M *P. signifer* (CON) Fed	NS
SL M *P. signifer* (CON) Unfed	NS
SL M *P. signifer* (EXPT) Fed	NS
SL M *P. signifer* (EXPT) Unfed	NS
SL F *P. signifer* (CON) Fed	P<0.0003
SL F *P. signifer* (CON) Unfed	P<0.0001
SL F *P. signifer* (EXPT) Fed	NS
SL F *P. signifer* (EXPT) UNFED	NS
SL M *G. holbrooki* (EXPT) Fed	P<0.0065
SL M *G. holbrooki* (EXPT) Unfed	NS
SL F *G. holbrooki* (EXPT) Fed	NS
SL F *G. holbrooki* (EXPT) Unfed	NS
TL M *P. signifer* (CON) Fed	P=0.0418
TL M *P. signifer* (CON) Unfed	NS
TL M *P. signifer* (EXPT) Fed	NS
TL M *P. signifer* (EXPT) Unfed	NS
TL F *P. signifer* (CON) Fed	P=0.0050
TL F *P. signifer* (CON) Unfed	P=0.0001

Table 4.8 continued.

Parameters	Significance
TL F *P. signifer* (EXPT) Fed	NS
TL F *P. signifer* (EXPT) Unfed	NS
TL M *G. holbrooki* (EXPT) Fed	P=0.0115
TL M *G. holbrooki* (EXPT) Unfed	NS
TL F *G. holbrooki* (EXPT) Fed	P=0.0001
TL F *G. holbrooki* (EXPT) Unfed	P=0.0072
WT M *P. signifer* (CON) Fed	P=0.0052
WT M *P. signifer* (CON) Unfed	P=0.0012
WT M *P. signifer* (EXPT) Fed	NS
WT M *P. signifer* (EXPT) Unfed	NS
WT F *P. signifer* (CON) Fed	NS
WT F *P. signifer* (CON) Unfed	P=0.0064
WT F *P. signifer* (EXPT) Fed	NS
WT F *P. signifer* (EXPT) Unfed	NS
WT M *G. holbrooki*(EXPT) Fed	P=0.0022
WT M *G. holbrooki* (EXPT) Unfed	P=0.0097
WT F *G. holbrooki* (EXPT) Fed	P=0.0001
WT F *G. holbrooki* (EXPT) Unfed	P=0.0001

KEY

CON: Treatment with *P. signifer* only
EXPT: Treatment with *P. signifer* and *G. holbrooki*
M: male
F: female
Sur: survival
SL: standard length
TL: total length
WT: weight
Fed: given supplementary food
Unfed: Not given supplementary food
NS: not significantly different

Table 4.9 Summary of tests on reproductive parameters at harvest (from page 146).

Parameter	Test	Significance
sig Fec Con Fed/Unfed	Kruskal-Wallis	NS
sig Fec Expt Fed/Unfed	Kruskal-Wallis	NS
sig Fec (Con Unfed)/(Expt Unfed)	Kruskal-Wallis, Dunn's post test	$P<0.01$
sig Fec (Con Fed)/(Expt Fed)	Kruskal-Wallis, Dunn's post test	$P<0.001$
sig Ovary Wt Con Fed/Unfed	Kruskal-Wallis	NS
sig Ovary Wt Expt Fed/Unfed	Kruskal-Wallis	NS
sig Ovary Wt (Expt Fed)/(Con Fed)	Kruskal-Wallis, Dunn's post test	$P<0.001$
sig Ovary Wt (Expt Unfed)/(Con Unfed)	Kruskal-Wallis, Dunn's post test	$P<0.01$
sig Testis Wt Con Fed/Unfed	One-way ANOVA	NS
sig Testis Wt Expt Fed/Unfed	One-way ANOVA	NS
sig Testis Wt (Expt Fed)/(Con Fed)	One-way ANOVA	NS
sig Testis Wt (Expt Unfed)/(Con Unfed)	One-way ANOVA	NS
M sig GSI Con Fed/Unfed	One-way ANOVA	NS
M sig GSI Expt Fed/Unfed	One-way ANOVA	NS
M sig GSI (Expt Fed)/(Con Fed)	One-way ANOVA	NS
M sig GSI Expt (Unfed)/(Con Unfed)	One-way ANOVA	NS
F sig GSI Con Fed/Unfed	Kruskal-Wallis	NS
F sig GSI Expt Fed/Unfed	Kruskal-Wallis	NS
F sig GSI (Expt Fed)/(Con Fed)	Kruskal-Wallis, Dunn's post test	$P<0.001$
F sig GSI (Expt Unfed)/(Con Unfed)	Kruskal-Wallis, Dunn's post test	$P<0.05$
G Fec Expt Fed/Unfed	Mann-Whitney	$P=0.0087$
G Testis Wt Fed/Unfed	Unpaired 2 tailed t-test	NS
G Ovary Wt Fed/Unfed	Mann-Whitney	$P=0.0090$
M G GSI Fed/Unfed	Unpaired 2 tailed t-test	NS
F G GSI Fed/Unfed	Unpaired 2 tailed t-test	$P=0.0007$

Key.

sig: *P. signifer* G - *G. holbrooki*
Con: treatment with *P. signifer* only
Expt: *P. signifer* and *G. holbrooki*
M: male
F: female
Fed: given supplementary food
Unfed: not given supplementary food
Fec; fecundity
Wt: weight
GSI: gonadosomatic index
NS: not significant

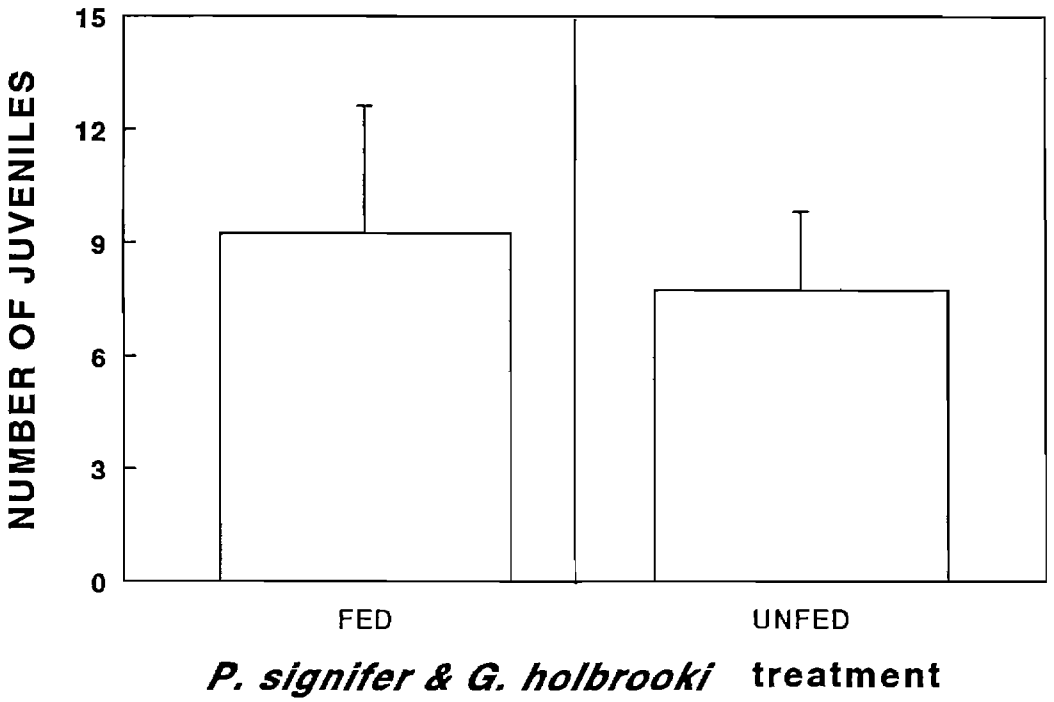


Figure 4.38 The mean number of individuals (mean ± SE, n=4) of juvenile *Gambusia holbrooki* at harvest in tanks with *Pseudomugil signifer* that were given supplementary food (FED) or not given supplementary food (UNFED).

4.3.3 Behavioural observations.

4.3.3.1 Behavioural observations I -interactions of P. signifer housed with and without *G. holbrooki*.

Table 4.10 summarises the main observations noted for *P. signifer* in the absence or presence of *G. holbrooki*.

Table 4.10 Observations of *P. signifer* with and without *G. holbrooki* from October 1992 to February 1993 in aquaria.

	Control tanks Without *G. holbrooki*	Experimental tanks With *G. holbrooki*.
Food capture	quick, active	reluctant, followed after *G. holbrooki*
Schooling	Yes	No, dispersed.
Aggressiveness	Slight by males during courting but no fin nipping	Female *G. holbrooki* particularly aggressive towards *P. signifer* sometimes pursuing until death.
Appearance after 11 weeks	Both sexes healthy. Females fat.	Emaciated, caudal fin damage.
Male colouration after 15 weeks	All extremely bright breeding condition.	1 bright, others not bright.
Eggs on java moss after 16 weeks	Present	Absent
Male *P. signifer* survival by harvest	6 out of 6 survived	2 out of 3 survived
Female *P. signifer* survival by harvest	11 out of 12 survived	0 out of 6 survived
Male *G. holbrooki* survival by harvest	not applicable	3 out of 3 survived
Female *G. holbrooki* survival by harvest	not applicable	5 out of 6 survived

G. holbrooki (in particular the females), were observed to nip the fins of *P. signifer*. Fig. 4.39 shows the caudal fin of an individual *P. signifer* from the control treatment (*G. holbrooki* absent) and an individual *P. signifer* from the experimental treatment (*G. holbrooki* present). In addition, this constant aggression of female *G. holbrooki*, mainly against female *P. signifer*, often resulted in the death of *P. signifer*.

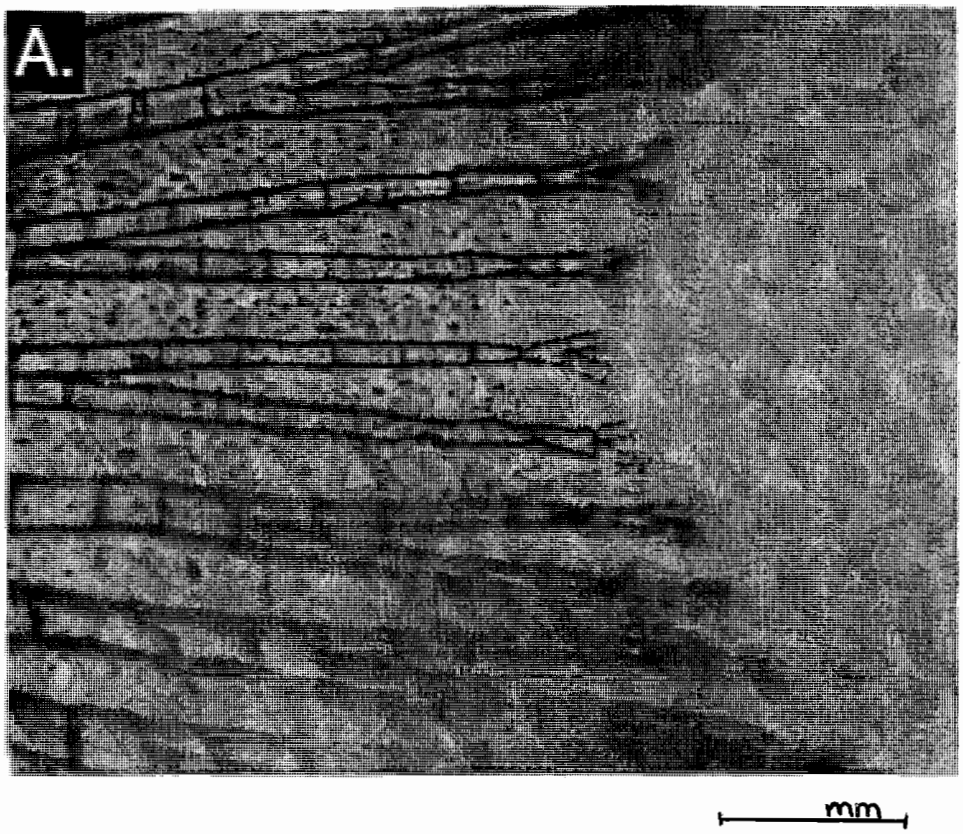


Figure 4.39 A. The caudal fin of *Pseudomugil signifer* from a control tank (no *Gambusia holbrooki*). Note the bifurcate appearance of the rays. The fin has an even edge.**B.** The fin of *P. signifer* from an experimental tank (housed with *Gambusia holbrooki*). No terminal bifurcation of the rays is evident and the edge of the fin is ragged, as it has been eaten away.

P. signifer from the control treatment tank had even-edged caudal fins with bifurcate rays. However, the caudal fin of the experimental *P. signifer* had a ragged appearance with no bifurcation of the rays. Female *G. holbrooki* were also observed to attack *P. signifer* on many occasions. A behavioural hierarchy was observed in which female *G. holbrooki* dominated overall (observed chasing adult *P. signifer*), followed by male *G. holbrooki* followed by male *P. signifer* (although their aggressive tendencies appeared to be directed towards territory guarding) and lowest in the hierarchy were female *P. signifer*, which suffered greatest mortality among adult fish (Table 4.10).

In addition very little or no colour development was observed on the fins of male *P. signifer* in the experimental tank compared with those in the control tanks. Colour development is an indication of spawning behaviour (Howe, 1987) and therefore as would be expected, no eggs were found, prior to the death of female *P. signifer* in the experimental tank at harvest.

Survivals of *P. signifer* and *G. holbrooki* are recorded in Table 4.10. In the control tank all but one *P. signifer* survived until harvest. In the experimental tank two of the three male *P. signifer* survived but no females survived.

4.3.3.2 Laboratory behavioural experiment II - interactions between adult *P. signifer* and *G. holbrooki* to larval *P. signifer*.

Within 10 minutes of the introduction of the adult *G. holbrooki* and *P. signifer* the females of *G. holbrooki* were actively hunting the young *P. signifer*. Within 20 minutes all of the juveniles had been eaten.

4.4 Discussion.

As detailed above two tank experiments were conducted in this study. The experiments were performed in two consecutive years (1992-1993) over the breeding period of *P. signifer* and *G. holbrooki*. In addition two behavioural observation studies were conducted to determine what was actually happening within the test tanks. All of these investigations were designed to assess whether *G. holbrooki*

threatened the growth and reproduction of *P. signifer*. The results of the studies indicated that the presence of *G. holbrooki* had significant effects on both the growth and fecundity of *P. signifer*.

4.4.1 Growth and behavioural patterns.

From the first experiment it was determined that the survival of *P. signifer* was significantly lower when housed with *G. holbrooki*. The surviving *P. signifer* housed with *G. holbrooki* did not grow in length or weight, indicating that there is a clearly detrimental effect of *G. holbrooki* on *P. signifer* when kept at these stocking ratios in confined environments. In addition, the surviving *P. signifer* in experimental tanks had caudal fin damage which was not observed in *P. signifer* from control tanks. Standard length may perhaps be a better indicator of growth than total length as any damage to the caudal fin would give a smaller total length measurement. No increase in total length of *P. signifer* was found in the presence of *G. holbrooki*, which could be at least partly attributed to the loss of the distal edges of the caudal fin due to nipping by *G. holbrooki*. Agonistic fin nipping behaviour was also displayed by *G. holbrooki* in laboratory aquaria, in the behavioural experiment, when the two species were housed together. It is possible that fin nipping only occurs when fish are crowded and therefore the tanks were overstocked. However such conditions occur in the field with receding tides or increased drought conditions high densities of fish can often be left in ponds or puddles for extended periods of time (pers obs.; Pen, 1990). Unmack and Brumley (1991) have also found caudal fin damage in *S. vermeilipinnis* in the wild where they occur in small ponds with *G. holbrooki*.

From the behavioural experiment, it was observed that control *P. signifer* swam as a school whereas when housed with *G. holbrooki* they did not school. Semple (1986) had also observed schooling behaviour by *P. signifer*. This schooling behaviour possibly provides security and when dispersed their stress levels could be greater leading to less growth.

Female *P. signifer* grew in weight and total length from introduction to harvest in the absence of *G. holbrooki*. In male *P. signifer* there was no growth in total length

from introduction to harvest but there was an increase in weight in the absence of *G. holbrooki*. It is clear that female *P. signifer* grow when not housed with *G. holbrooki*. The effect is not as clear in male *P. signifer* which could possibly be because they had reached their maximum size.

4.4.2 Water quality.

It appears that there have been no systematic experiments to determine optimum water conditions for survival, growth and reproduction in *P. signifer*. The literature only covers information on satisfactory aquarium conditions (Merrick and Schmida, 1984) and a range of temperatures and Ph values in which the fish have been found in the field.

G. holbrooki can survive in a wide range of salinities (Dutta Gupta *et al.*, 1991) and temperatures (Meffe, 1992). They are also known to survive in conditions of low dissolved oxygen (Sjogren, 1972) and general poor water quality (Bay, 1967; Grant, 1978).

Water quality differed between the tanks in the outdoor experiments, but not sufficiently to affect the overall results.

4.2.2.1 Temperature.

The temperature ranges found in the current experiments were within the range that *P. signifer* (Allen and Cross, 1982, Merrick and Schmida, 1984) and *G. holbrooki* (Otto, 1973) are found in the field. There were no significant differences in temperature levels between control and experimental treatments, and in both experiments rising water temperatures were recorded over time, consistent with ambient temperatures. It has previously been found that spermatogenesis and oogenesis in *P. signifer* were at their peak in the summer months with the increase in temperature under field conditions (Chapter 3 and Howe and Howe, 1991) and the tank experiments confirmed these results.

4.4.2.2 pH.

Even though the pH levels in these experiments were higher than has previously

been found in the field for *P. signifer*, it is suggested that the pH levels were not detrimental as they were short term experiments, and photosynthesis by aquatic plants can cause the pH value of the water to exceed 9.0 (Alabaster and Lloyd, 1982; Piper *et al.*, 1982). Current field studies (Chapter 5) have found *P. signifer* at Deep Creek at a pH of 8.1. No correlation was found between pH at harvest and the proportion of *P. signifer* surviving to harvest and no differences could be identified between experimental treatments.

4.4.2.3 Dissolved oxygen.

No differences in dissolved oxygen concentrations between control and experimental groups could be shown in either experiment and further, it was seen that low and variable dissolved oxygen concentrations in the experimental tanks at harvest in the first experiment did not appear to detrimentally affect *P. signifer*.

4.4.2.4 BOD.

As stated in the results there was variation in BOD values at introduction in the second experiment between treatments but they were all low and therefore considered to be of acceptable water quality.

In both experiments the BOD values had increased by harvest. However *P. signifer* that were housed in the control tanks still grew in length and weight, indicating that the water quality was adequate for this species, which tolerates polluted water (Grant, 1978). There was no significant difference between the BOD values in the control and experimental tanks at harvest, therefore any differences between the two treatments in terms of numbers, lengths or weights of *P. signifer* should not relate to the BOD levels.

4.4.2.5 Ammonia, Nitrate and Hardness.

Despite the pH values found in both tank experiments being relatively alkaline, un-ionised ammonia levels did not become excessive in summer. In the first tank experiment un-ionised ammonia concentrations fluctuated but remained below suggested acceptable limits (Cook, 1986) throughout. Likewise, in the second tank

experiment the un-ionised ammonia at harvest was greater than at introduction but was well below acceptable levels.

At no time did nitrate concentrations reach levels that might be acutely or chronically detrimental to fish (Reddacliff, 1985; Langdon, 1988).

Hardness in these experiments fell within an acceptable range for fish (Langdon, 1988).

For ammonia, nitrate and hardness there were no identifiable differences between treatments in either experiments.

4.4.2.8 Invertebrates and phytoplankton.

The experimental tanks were colonised by a reasonable variety of invertebrates and phytoplankton. Mosquito larvae were not present at harvest in control or experimental ponds indicating that both species successfully eradicated them. The number of invertebrates and phytoplankton and the general appearance of the water (slightly turbid) suggested that productivity was adequate for food production for the species. Food did not appear to be a limiting resource in either experiment as rotifers and cladocerans were abundant. Although a weak but significant correlation was found between numbers of rotifers at harvest, the proportion of female *P. signifer* surviving to harvest, no differences were found between fed and unfed groups of *P. signifer.*

4.4.3 Physiological mechanisms of growth inhibition.

The reason that growth was more pronounced in female than male *P. signifer* could be related to the reproductive physiology of the fish. In the absence of *G. holbrooki*, normal growth of the ovary and hence overall body weight could be a more important event than the growth of the testes in the male *P. signifer.* In fish, eggs represent a larger energetic investment than sperm, and this investment, chiefly in yolk production, is critically important to the survival of the developing young (Jobling, 1993). In addition, the GSI was significantly greater in the female *P. signifer* when *G. holbrooki* was absent, whether or not supplementary food was provided, which supports this argument. In the case of the male *P. signifer*, this was

true only with supplementary feeding.

When ovarian weights and fecundities were plotted against body weight for the four treatment groups, it was surprising to find that only one group showed evidence of a relationship between body size and fecundity. It was perhaps not so surprising in the case of the *P. signifer* in the experimental groups, as almost all had a fecundity of zero, and very low ovarian weights. In the control groups, only the unfed group showed significant relationships between body weight and fecundity, and body weight and ovarian weight. The supplementary fed control group showed no such relationship, and this appears due to the smaller fish in this group having greater fecundity and ovarian weight (on inspection of Fig. 4.27A and B, and Fig. 4.28A and B). It would be interesting to specifically examine this effect in a sufficient number of animals, as this may represent a subtle feeding effect in the female. It is, however, completely swamped in this experiment by the detrimental effects of the presence of *G. holbrooki*.

The females possibly require a less stressed environment (minus *G. holbrooki*) for breeding and growth whereas the males are not as seriously affected by the presence of *G. holbrooki*, as they may react differently to the same stress. Male *P. signifer* were observed to be aggressive during the behavioural study and may therefore resist attack by *G. holbrooki*. In the behavioural experiment no female *P. signifer* survived to harvest, which lends support to this view. *P. signifer* males are definitely territorial during this breeding period (pers. obs.) and this could lead to aggressive behaviour. The amount of metabolic effort expended in the production of large eggs far outweighs that required for the production of sperm. Limited observations available suggest that female *P. signifer* in the wild may live for only one breeding season, that is, under normal circumstances they grow for 12-18 months until death (pers. obs.). On the other hand, the males may live for two years or more (pers. obs.) and therefore could have attained their maximum length and weight at the beginning of the experiment. This breeding strategy is exemplified by *Antechinus stuartii* males which only live for one year, dying after a brief breeding season, whereas females live more than one year (Lee *et al.*, 1982). Krumholtz (1948) and Pen (1990) suggest that *G. holbrooki* die in the summer in which they

reach maturity. They do not, however, distinguish between the length of life in males and females. It is unclear whether female poeciliids live longer than males because of the paucity of good age structure data (Snelson, 1989).

Indications of reproductive success were obtained from the first experiment when *P. signifer* produced young in the absence of *G. holbrooki* but produced none when housed with *G. holbrooki*. It would appear that the lack of young *P. signifer* in experimental tanks was not due to the eggs being eaten but caused by lack of development of the ovary as was demonstrated by the second experiment. The first experiment demonstrated that there were possible reproductive effects of *G. holbrooki* on *P. signifer*. It was determined that, given appropriate time through the breeding season, young *P. signifer* survived in control tanks but not in the tanks where *P. signifer* cohabited with *G. holbrooki*. The harvest in February allowed the eggs time to hatch.

The purpose of the second experiment was to determine whether the fecundity of *P. signifer* was affected by *G. holbrooki*. December was an appropriate month for fecundity measurements as determined in Chapter 3 and by Howe and Howe (1991). Although no juvenile *P. signifer* were found in control tanks at harvest, eggs were present in all control tanks. In this experiment the fecundity was significantly affected by the presence of *G. holbrooki* using a one to one species ratio, a one to two male to female sex ratio and a stocking rate of 180 fish per cubic metre. *P. signifer* housed with *G. holbrooki* in the current experiments could not be considered fit from a Darwinian perspective as fitness of fish is related to survival and fecundity (Calow, 1985). It would be of great value to repeat these experiments with different species ratios, sex ratios and stocking levels.

4.4.4 Possible reasons for decline in reproductive activity of *P. signifer* when housed with *G. holbrooki*.

The question remaining unanswered, is what is actually governing the reduction in ovarian weight, GSI and fecundity in *P. signifer* housed with *G. holbrooki*? The experiments to date indicate that fecundity and growth of *P. signifer* are reduced when the fish are housed with *G. holbrooki*. Further investigation into the processes

involved would be valuable.

When neither hybridisation between native and exotic species, nor predation are involved, interspecific competition is usually implicated as a mechanism of interaction (Schoenherr 1981). *G. holbrooki* is an aggressive species and a fin-nipper (Innes 1966, McDowall 1980). When aggressive behaviour affects the survival potential of reproductively active females the interaction has been interpreted as a form of interspecific competition for space (Schoenherr 1981). This mechanism could be operating under the confined conditions of this experiment.

4.4.4.1 Interruption of normal behaviour patterns.

The normal behaviour of *P. signifer* was disrupted by *G. holbrooki* which in turn could result in physical stress leading to endocrine responses and lower reproductive activity which could be exacerbated or triggered by starvation. Stress of an individual has been considered the result of a disturbance which can reduce survival (Brett, 1958). "The primary effects of stress occur in the endocrine system which, in turn, mediates the secondary effects characterised by metabolic changes in organ systems as the animal attempts to maintain homeostasis" (Ellis, 1981). Stress has been considered by Pickering (1981) as an integrated response of primary (neural and neuro-endocrine), secondary (the physiological consequences of primary responses) (Mazeaud *et al.*, 1977) and tertiary changes (e.g. changes in behaviour, decreased growth rate or increase in disease) (Wedemeyer and McLeay, 1981). The direct agonistic behaviour of *G. holbrooki* could cause physiological stress leading to regression of ovarian function, and reductions in feeding and metabolic processes.

4.4.4.2 Endocrine response.

It is also possible that a subtle effect such as pheromone production by *G. holbrooki* might cause changes in *P. signifer*'s endocrine system. It has been shown that fish use their hormones and metabolites as pheromones (Stacey *et al.*, 1987). Quite distantly related species of fish can respond to chemical alarms from each other (Mathis and Smith, 1993). Teleosts have a working hypothalamic-pituitary-interrenal axis which responds to stress (Donaldson, 1981). Changes in the

adrenergic function of fish have been demonstrated by high concentrations of catecholamines in fish plasma (Fontaine *et al.*, 1963). Elevation of catecholamines can be the consequence of stress in many fish species (Mazeaud *et al.*, 1977). However, this elevation can also occur from other causes and catecholamines are notoriously difficult to measure (Mazeaud and Mazeaud, 1981).

The responses to stress are mediated by the hypothalamic-pituitary-adrenocorticoid axis resulting in the stimulation of the adrenal cortex and a rise in serum corticosteroids (Fletcher, 1981). It would be of interest to measure the level of cortisol in *P. signifer* when housed with or without *G. holbrooki*. Cortisol, like catecholamines is difficult to measure meaningfully as capture stress itself causes elevation of plasma cortisol (Sumpter *et al.*, 1987; Safford and Thomas, 1987,). To resolve this problem live fish could be snap frozen, or sampling could be performed efficiently (with as little disturbance as possible) with anaesthetised fish (Pickering and Pottinger, 1987). Stress appears to be an important event in the reproductive decline and lack of growth of *P. signifer* when housed with *G. holbrooki*. Stress has been postulated to affect the reproductive axis of trout (either by stress hormones (ACTH and cortisol) acting directly on the gonads, or at the pituitary and/or hypothalamus (Sumpter *et al.*,1987). It has been determined that stress can suppress somatic growth in teleost fish (Pickering, 1990) indicating that a stress mechanism is operating on *P. signifer* when housed with *G. holbrooki*. Vijayan and Leatherland (1990) found that high stocking densities affect cortisol secretion in brook charr, *Salvelinus fontinalis*.

4.4.4.3 Physiological stress.

Stress is "the effect of any force which tends to extend any homeostatic or stabilising process beyond its normal limit, at any level of biological organisation" (Esch *et al.*, 1975). It was determined by Pickering and Pottinger (1987) that haematological changes which indicate the state of the fish's defence systems provide more accurate account of stress in salmonids than the hypothalamic-pituitary-interrenal axis. Stress affects the osmotic and ionic regulation in fish (Eddy, 1981). The body fluids of fish must be kept under control during intervals of growth and

reproduction (Eddy, 1981).

G. affinis caused physiological stress to *Poeciliopsis occidentalis* (top minnow) adults by constant aggression resulting in reduced fecundity, cessation of feeding and increased mortality of this fish (Schoenherr, 1981). From both the behavioural aquarium experiments and outdoor tank experiments in the present study it is suggested that the agonistic behaviour of *G. holbrooki* to *P. signifer* caused stress which was at least partially responsible for the decline in growth rates and fecundity of *P. signifer* when housed with *G. holbrooki*.

In addition, there was possibly direct competition for shelter and space.

4.4.4.4 Stocking density.

The results could partially be related to the stocking density. In studies on salmonid fish crowding, it was shown that there was a transient plasma cortisol elevation and a protracted transformation in the circulating blood cells (Pickering and Pottinger, 1987). In the wild, *P. signifer* population densities range from less than 1 fish per cubic metre to several hundred per cubic metre (pers. obs.). They generally school unless involved in spawning behaviour where much smaller numbers of the species are found in the same area. Fish in the wild generally have the option to seek larger expanses of water. There are however, some species, for example the redfinned blue eye (*S. vermeilipinnis*), where fish have been found in small shallow ponds together with *G. holbrooki* and are therefore restricted in movement (Unmack and Brumley, 1991).

It would have been ideal to have run these experiments with a range of stocking densities. Nevertheless the information obtained from this study provides some baseline data which could be expanded with further experiments. The results clearly establish that at least under these conditions of density, the presence of *G. holbrooki* is very detrimental to the survival growth and reproduction of *P. signifer*. These results have clear conservation implications. For example, *P. mellis* is endangered and *S. vermeilipinnis* is threatened in the wild (Jackson, 1994). The added complication of *G. holbrooki* sharing their habitat could contribute to their demise.

4.4.4.5 Territoriality.

Male *P. signifer* are territorial (observed in the field during the present study and in aquaria by Semple, 1986) and *G. holbrooki* could have displaced them from their territory. In the behavioural experiment it was clearly observed that the *G. holbrooki* dispersed schooling *P. signifer*. In addition, agonistic behaviour by *G. holbrooki* was frequently observed.

It is undoubtedly one or more of the above factors that ultimately leads to the reduced activity and size of the ovary of female of *P. signifer*.

4.4.5 Food as a limiting resource in the test tank experiments of *P. signifer* when housed with *G. holbrooki*.

Booth (1980) presented data on the stomach contents of both *P. signifer* and *G. holbrooki*. The major food types consumed by both species were insects followed closely by polychaetes (Booth, 1980). Both species of fish are known to have a comparable diet and react to prey at a similar distance (Booth, 1980).

The question arose after the first experiment as to whether food was a limiting resource in the lack of growth of *P. signifer* when housed with *G. holbrooki* as no additional food was provided. It is not known whether *G. holbrooki* might outcompete *P. signifer* for limited food resources. The food that was added consisted of insects and crustaceans, supplemented with dried food when these were unavailable.

In female *P. signifer*, feeding in the absence of *G. holbrooki* (controls) was associated with increased numbers of female *P. signifer* surviving. This was the only occasion when any association with feeding was observed in either sex, as measured by changes in total length, standard length and weight. Paradoxically, in female *P. signifer* there was an increase in weight in controls with no supplementary feeding but no increase in weight in control *P. signifer* that were given supplementary feeding. These findings emphasise the fact that supplementary feeding provides no assistance in weight gain. There was sufficient natural food in the experimental system and therefore food was not a limiting resource.

4.4.6 Effect of food restriction on *G. holbrooki*.

There was a feeding effect in female *G. holbrooki* based on reproductive measurements. The ovarian weights, fecundity and GSI of female *G. holbrooki* increased when given additional food compared with fish in tanks that were not given additional food. This suggests some food restriction was involved although food organisms were present. This effect was not observed among the males under regimes of supplementary feeding. This may reflect the greater requirement for resources involved in the production of eggs.

4.4.7 Possible reasons for the difference in response of the two species to supplementary feeding.

The facts that reproductive activity of female *G. holbrooki* was restricted when additional food was not given, and that *P. signifer* did not seem to be affected in this manner, or was more subtly affected, could be due to several factors. First the two species have different modes of development. *G. holbrooki* may require additional food as it requires a large amount of energy to produce live young and in so doing increases its weight significantly during the breeding season. Mejen (1940), cited in Kamler (1992), found that under good trophic conditions oocytes situated near blood vessels were supplied with adequate nutrients. When food is decreased nutrient flow to oocytes near small blood vessels is restricted, resulting in an increase in variability and a decrease in average size of spawned eggs. Townshend and Wootton (1984) showed that the vitellogenic condition of oocytes in *Cichlasoma nigrofasciatum* could be related to the amount of food provided. *P. signifer* in the additionally fed tanks cohabiting with *G. holbrooki*, were neither better nor worse off than those in the unfed tanks with *G. holbrooki*. It might have been expected that the *P. signifer* in the unfed experimental tanks would be worse off than their fed counterparts, if *G. holbrooki* females had required more food as indicated by their reproductive condition in the tanks where supplementary food was provided. If the absence of sufficient food was a significant factor, *G. holbrooki* may have been expected to place even more competitive pressure upon the *P. signifer* in the unfed treatment. This did not appear to have occurred, and suggests that other factors such

as aggression may be involved rather than food. The suggestion that fecundity and ovarian weight may be increased in smaller fish, in the supplementary fed control tanks, is a subtle effect, and completely overshadowed by the deleterious effect of the presence of *G. holbrooki*.

4.4.8 How exotic species may affect native species.

In the introduction, two hypotheses were discussed which seek to explain to explain the effect of exotics on native species analagous to fish in the western USA and Australian fish. Firstly, it was suggested by Douglas *et al.*, (1994) that environmental degradation eliminates native fish allowing introduced species to replace them. Alternatively, direct replacement of the native by the introduced species may occur. The results of this study would lend support for the latter hypothesis in this case (under confined conditions) because in the experimental tanks *G. holbrooki* were able to grow and reproduce at the expense of *P. signifer*.

4.4.9 Replacement mechanisms.

Meffe (1985) made an assessment of replacement mechanisms as documented by Minckley (1973) and Schoenherr (1981) that could be involved with the replacement of *Poeciliopsis occidentalis* with *G. affinis*.

The more plausible mechanisms are discussed in terms of the relationships between *G. holbrooki* and *P. signifer*.

4.4.9.1 Parasites and disease.

G. holbrooki may have diseases or parasites that can decimate native species (Elton, 1958). No parasites were observed on *P. signifer* although they did have a bedraggled appearance after being housed with *G. holbrooki* and in this state could be more susceptible to disease, especially nonspecific secondary fungal infections (Reddacliff, 1985 and Mazeaud *et al.*, 1977).

4.4.9.2 Hybridisation.

P. signifer and *G. holbrooki* did not hybridise. As pointed out in the introduction,

the two species of fish are not sufficiently related for hybridisation to be remotely likely.

4.4.9.3 Competition.

Competition for space and possibly food, in addition to predation, was suggested by McIntosh *et al.*, (1992) to cause the decline of *Galaxias vulgaris* in the presence of *Salmo trutta*. The two species of fish in the present study are found in the same habitats in the Sydney region, and both are carnivores eating similar foods (Booth, 1980 and Booth *et al.*, 1985). No direct evidence was found that there was competition for food in the main tank experiments. However in the accompanying behavioural observations which were carried out at the same time as the first tank experiment, *G. holbrooki* were observed to be the first to eat the provided food. Competition for spatial resources can be surveyed by the addition or removal of one species, as executed by Fausch and White (1981). Competition for space must at least be implicated even though it may not be the only reason for reduced growth rates in *P. signifer*.

In addition, as discussed previously the direct aggressive behaviour by *G. holbrooki* appeared to affect the potential of female *P. signifer* to reproduce and this type of interaction has been interpreted as a form of interspecific competition for space (Schoenherr 1981).

4.4.9.4 Predation.

In the laboratory *G. holbrooki* were observed to actively fin nip and sometimes pursue *P. signifer* until death. When larval *P. signifer* were presented to adult *G. holbrooki* and *P. signifer* they were eaten within minutes by *G. holbrooki* even when substantial hiding places were available. Recent studies have implicated predation, not competition, in interactions of *G. holbrooki* with closely related species (Belk and Lydeard, 1994 and Schaefer *et al.*, 1994). However, the results of the present tank study cannot be explained by predation of *G. holbrooki* on *P. signifer* although predation as a mechanism may play some part. The most significant effects were observed in reduced growth and fecundity of *P. signifer* when housed with *G.*

holbrooki, not lack of survival. Stress and competition for space on *P. signifer* by *G. holbrooki* are at least partially responsible for the observed effects.

4.4.10 Future research and concluding remarks.

Further tank experiments are clearly needed, using different ratios of one species to another, sex ratios, and stocking numbers. Further investigation with stocking numbers could provide answers to the factors operating in competition for space. McIntosh *et al.* (1992) in their experiments with the exotic brown trout (*Salmo trutta* L.) and the native galaxiid (*Galaxias vulgaris* Stokell) suggested that spatial competition combined with other factors such as predation could explain the decline in numbers of the galaxiid. More than one factor is probably implicated in the effect of *G. holbrooki* on *P. signifer*. In addition to further exploration of stocking numbers and ratios of each species, other mechanistic behavioural studies would be of great value. These studies would benefit from being carried out both in the field and laboratory.

The aim of the current research has been to determine the effect of *G. holbrooki* on *P. signifer*, which could have major implications for other *Pseudomugil* species that live in more restricted habitats and are endangered. It was not designed as a competition experiment, but rather a study of the environmental impact of an introduced species on a native one. Therefore the effect of *P. signifer* on *G. holbrooki* has not been dealt with here in any detail. Other experiments in the future could look at this aspect, however.

The ability to produce large numbers of live young during a single breeding season, early sexual maturity, aggressiveness and tenacious hardiness (in their capability to live under many different conditions) make *G. holbrooki* a formidable fish to many of our small natives. More extensive experimentation is needed to elucidate the mechanisms by which *G. holbrooki* are able to reduce the growth and reproductive capacity of *P. signifer*. In addition, experiments could be designed both in the field and laboratory (in varying sized ponds) to determine whether similar trends in growth and reproductive retardation occur when *G. holbrooki* are housed or found with other small Australian native fish.

Pen (1990) and Pen and Potter (1991) in field experiments in a temperate river in Western Australia found no evidence that *G. affinis* either ate the eggs or young of the four indigenous fish that they coexisted with. Neither did they find any agonistic behaviour by *G. affinis* towards the other species. However, *G. affinis*, unlike the other four native species studied by these researchers, had a breeding season in summer. Perhaps the aggressiveness of *G. affinis* is only forthcoming in their own breeding period and therefore they do not have an impact on other native species that spawn in the spring. Predation of *G. affinis* on plankton communities has been noted to be less in winter than in summer (Hurlbert and Mulla, 1981). In addition, the native species in Pen and Potter's (1991) study were larger than *G. holbrooki*. *G. holbrooki* may only attack fish of a smaller size than themselves. However it has been reported that the survival of fingerling (28mm total length) of a large native species, the golden perch, *Macquaria ambigua* was reduced by fifty percent when stocked in dams with *G. holbrooki* (Barlow and Bock, 1981). These researchers also reported caudal fin damage of fingerling *Macquaria ambigua* when in dams with *G. holbrooki*.

Unmack and Brumley (1991) have found caudal fin damage in *S. vermeilipinnis* in the wild where they co-exist in small ponds with *G. holbrooki*. *S. vermeilipinnis* is a small fish, only reaching a maximum total length of 26mm (Ivantsoff *et al.*, 1991). Other explanations for the difference in observations on *G. holbrooki* by Pen and Potter (1991) and Unmack and Brumley (1991) could be that in the latter study both species had similar breeding periods, and there was a lack of space available for escape by the indigenous species. *S. vermeilipinnis* is found in very small ponds with little space available to escape from *G. holbrooki* whereas in the Collie River the indigenous species could escape. The conditions in which *S. vermeilipinnis* were found would be relatively similar to those in the present experiments.

The common *Gambusia* species have been recognised as dangerous fish to introduce outside of its natural distribution (Myers, 1965). From these tank studies it would appear that the growth and reproduction of *P. signifer* is affected by the presence of *G. holbrooki* under restricted space conditions. If *G. holbrooki* were to have a similar influence in the wild they could be considered as a major pest as they

have a wide natural range and have been deliberately or accidentally transferred to many parts of Australia. In the next section of research a pilot field study of four sites in the Sydney region have been examined to determine whether *G. holbrooki* has any effect on the number, length, weight and fecundity of *P. signifer* in the wild.

Chapter 5.

Pilot Study: Field investigation of effects of habitat conditions and presence of *Gambusia holbrooki* on *Pseudomugil signifer*.

5.1 Introduction.

The common species of *Gambusia holbrooki* and *Gambusia affinis* have been recognised as very dangerous fish to introduce to areas outside their natural distributions (Myers, 1965). It has been suggested that *G. holbrooki* has displaced a number of small native species of fish in Australia (Myers, 1965; Weatherley and Lake, 1967; Rolls, 1969; Otto, 1973; Gerking *et al.*, 1975; Tilzey, 1980; Kailola, 1981; Allen and Cross, 1982; Arthington *et al.*, 1983 Cadwallader and Backhouse, 1983; Lloyd *et al.*, 1986 and Wager and Jackson, 1993). The majority of evidence is circumstantial although a field study on an urban creek in Brisbane, Queensland suggested that *P. signifer* were affected more by habitat alteration than by the presence of large populations of *G. holbrooki* (Arthington *et al.*, 1983). The authors recommended further research in the field and laboratory to understand the nature and impact of interactions between native and introduced species.

Under tank conditions, documented in the previous chapter, it was found that the growth and reproduction of *P. signifer* were significantly affected by the presence of *G. holbrooki*. These experiments were conducted in a confined space in artificial tanks. In the first study reported in Chapter 4, it was found that no young *P. signifer* were produced when *G. holbrooki* were present. The second study in Chapter 4 was in part designed to determine whether this was due to predation on the eggs and young, or to effects on the reproductive physiology of the adults. It was therefore of interest to determine whether *G. holbrooki* could be shown to have a similar influence on *P. signifer* in the wild. If *G. holbrooki* were found to have a detrimental effect on *P. signifer* in the wild, they could be considered a major threat to Australian pseudomugilids as they have a wide habitat and distribution range and have been deliberately transferred to many parts of Australia (McKay, 1984).

A pilot study of four sites in the Sydney region (Homebush Bay, Narrabeen, Lane Cove River and Mooney Mooney Creek) was carried out to determine whether *G. holbrooki* has an impact on the number, length, weight and fecundity of *P.*

signifer in the wild. Two of the sites had only *P. signifer* (Lane Cove and Mooney Mooney Creek) and the other two sites were known to have sympatric populations of *P. signifer* and *G. holbrooki* (Homebush and Narrabeen). This study was conducted in parallel with the harvest period of the second tank study, in order to compare the results of the two, especially with respect to fecundity.

The main focus of the pilot study was on fecundity in *P. signifer* during the breeding period. It included an introductory investigation into whether fecundity, numbers and size of *P. signifer* were affected by habitat conditions (specifically pH, temperature, salinity, dissolved oxygen, un-ionised ammonia and turbidity) as well as by the presence of *G. holbrooki*. The results of this pilot field experiment were to be compared with the more comprehensive tank experiments which were harvested at the same time in December, 1993. This study was intended to follow up on the field studies that were carried out in Chapter 3. It was anticipated that this pilot experiment would be followed by a much more extensive field study over a succession of seasons. The sampling was carried out two months beyond the steep rise in ovarian weight as indicated in Figure 3.4, Chapter 3, when the fish would still be reproductively active.

5.2 Materials and Methods.

5.2.1 Site selection.

In the field study of reproductive activity of *P. signifer* over a twelve month period (Chapter 3), no *G. holbrooki* were caught with the *P. signifer* at the Lane Cove River site whereas *G. holbrooki* were caught on numerous occasions at Deep Creek, Narrabeen. The Lane Cove and Narrabeen sites were therefore selected for this pilot study as they fitted the criteria for the experiment and relevant data had been gathered monthly for one year (February 1985 to January 1986) on the reproductive activity of *P. signifer*. Collections at Narrabeen were conducted at both Deep Creek and Middle Creek (unlike collections for the studies performed in Chapters 3 and 4 which only used Deep Creek). In this chapter the site is referred to as Narrabeen. In discussions with several staff from the NSW State Fisheries it was suggested that Mooney Mooney Creek would yield another site having *P. signifer* but no *G. holbrooki*. A preliminary survey carried out in November, 1993 found no

G. holbrooki. It was previously ascertained through random sampling by the author from 1988 to 1991 that both *P. signifer* and *G. holbrooki* inhabited the Homebush Bay area. Sampling in this study was conducted over a nine day period from 11th to 19th December, 1993. The second tank experiment (Chapter 4), with which the results of the pilot field study were to be compared, was harvested on 20th and 21st December, 1993.

5.2.2 Site descriptions:- Lane Cove and Narrabeen.

Fig. 5.1 is a map showing the location of the four sites used in this study. Descriptions of the Lane Cove and Narrabeen sites with their grid references are detailed in Chapter 3. Fig. 5.2 is a location map of the eight stations sampled at the Lane Cove River site.

Fig. 5.3A shows sampling at station 1, which was a small sandy beach with shallow water surrounded by stands of the mangrove *Avicennia marina*. Schools of *P. signifer* were clearly observed swimming in the water. This station yielded the largest catch of *P. signifer* at Lane Cove. The absence of snags meant that capture was less difficult than at other stations. Fig. 5.3B shows station 6 at Sugar Loaf Bay, Lane Cove. The substrate at this station was composed of a firm mud and there were numerous snags in the water. *A. marina* pneumatophores and trees lined the area. *P. signifer* were caught at this station but the presence of snags made it difficult to catch large numbers.

Fig. 5.4 is a location map of the eight stations used for sampling at the Narrabeen site. Fig. 5.5 shows two photographs typical of the Narrabeen site. Fig. 5.5A shows station 3 where *P. signifer* but not *G. holbrooki* were caught. Reeds and long grass lined the fore edge of the river bank with *Casuarina* sp. behind. Fig. 5.5B (where both *P. signifer* and *G. holbrooki* were netted) shows station 4 which was half way along Waterfall Creek. *Casuarina* sp. and reed beds lined one bank whilst *Lantana camara*, a declared noxious weed in New South Wales, was well established on the other side of the creek.

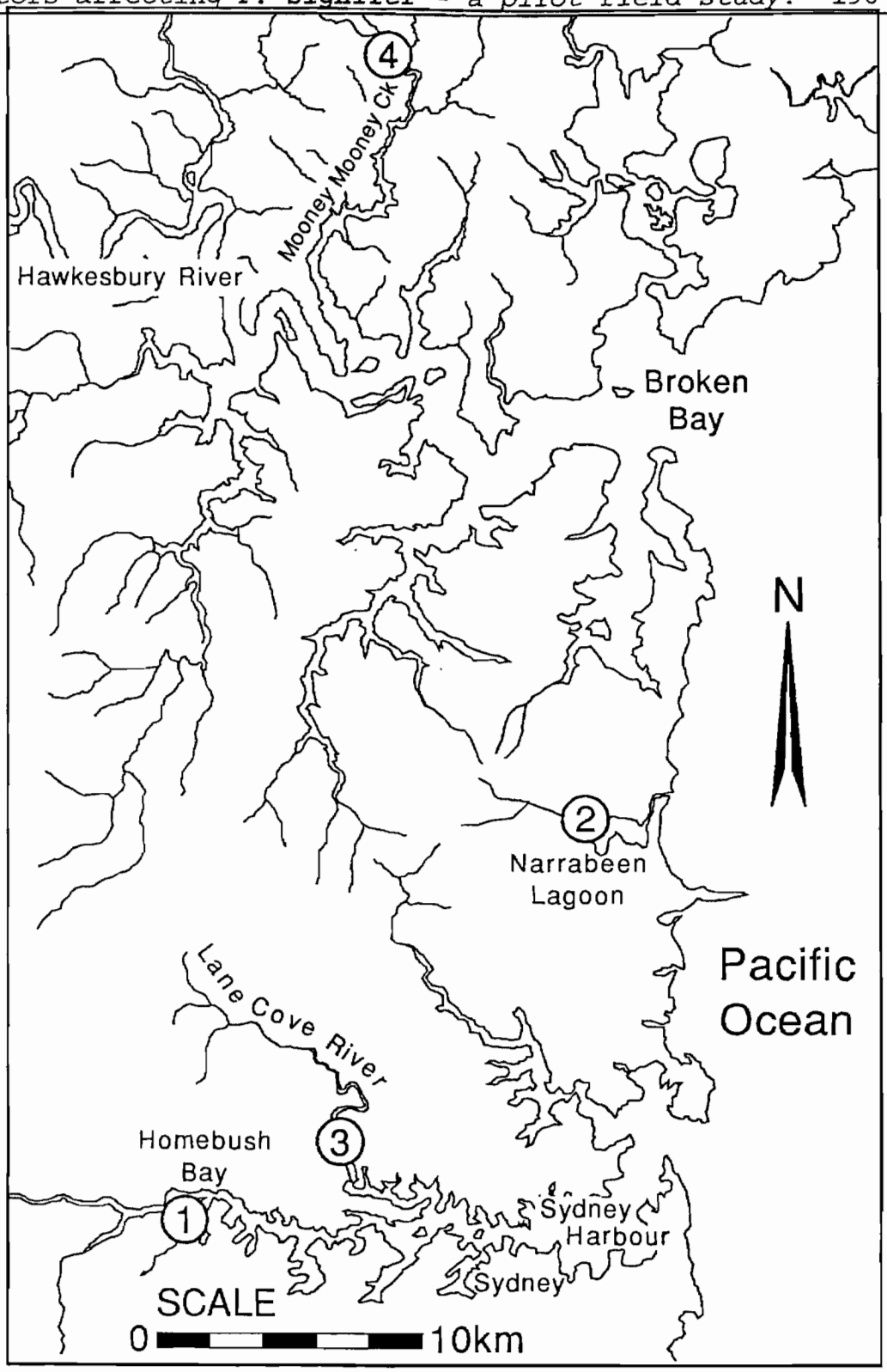


Figure 5.1 Location of the four sites (Homebush Bay (1), Narrabeen (2), Lane Cove River (3) and Mooney Mooney Creek (4)) used for study in December, 1993.

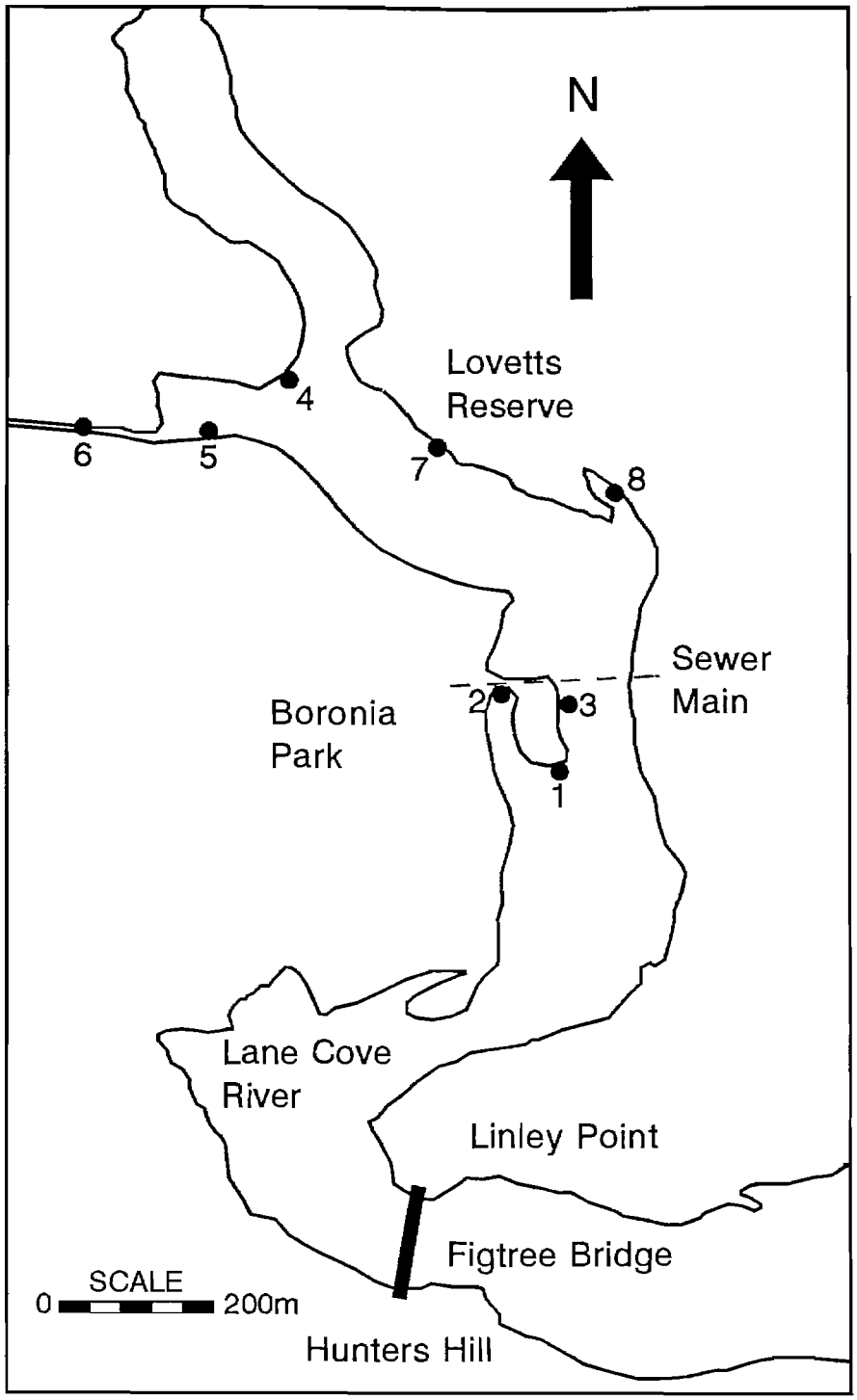


Figure 5.2 Location of the eight stations used for sampling *Pseudomugil signifer* at Lane Cove River (Site 3 Figure 5.1) in December, 1993.

A

B

Figure 5.3 (A) Sampling at Station 1 at the Lane Cove Site, a small sandy beach surrounded by stands of *Avicennia marina* in December, 1993. (B) Station 6 at Lane Cove showing the numerous snags and surrounded by the mangrove *Avicennia marina* in December, 1993.

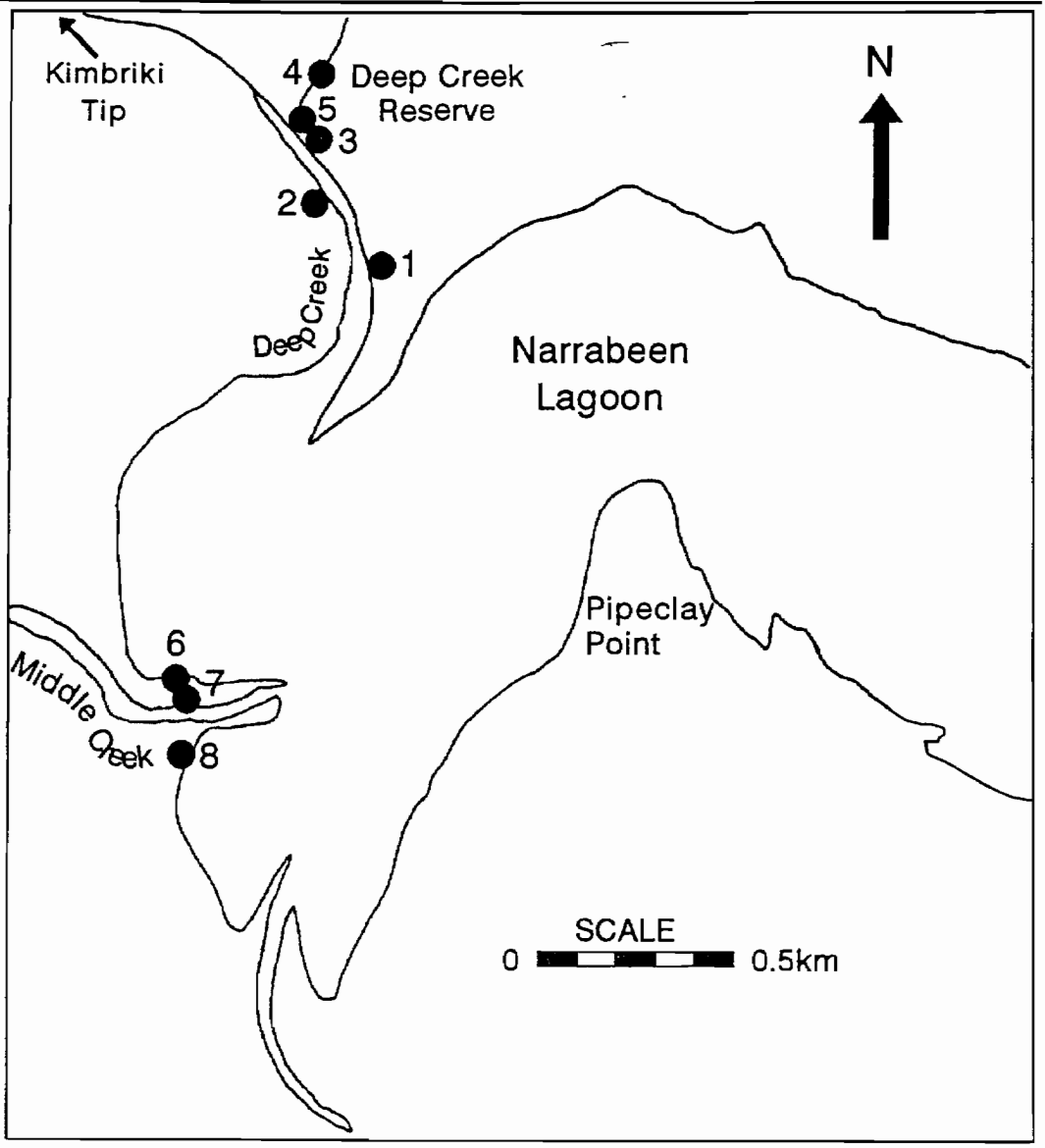


Figure 5.4 Location of the eight stations used for sampling *Pseudomugil signifer* and *Gambusia holbrooki* at Narrabeen (Site 2 in Figure 5.1) in December, 1993.

A

B

Figure 5.5 (A) Station 3 at Narrabeen where *Pseudomugil signifer* but not *Gambusia holbrooki* were caught in December, 1993. (B) Station 4 at Narrabeen where both *P. signifer* and *G. holbrooki* were caught in December, 1993.

5.2.2.1 Homebush Bay.

The sampling site was situated approximately twenty kilometres from the mouth of Sydney Harbour. Two hundred years ago Homebush Bay was larger and dominated by estuarine wetlands (saltmarshes, tidal creeks, mudflats and mangroves). There was a shale woodland on its outer edge. For several decades the area has been used for industrial development (brick works, abattoirs and chemical factories), which has nearly eradicated the shale woodland from the bay. The reclamation of land has altered the distribution of the mangroves. The area is now composed of reclaimed man made fill, of dredged estuarine sand and mud, demolition rubble and industrial and household waste. (Anon, Bird Survey, 1989). Homebush Bay contains the largest stand of the grey mangrove, *A. marina*, remaining in Sydney Harbour. Mangroves line the bay. Plantings of *A. marina* have been made by the Bicentennial Park Authority since 1988.

Fig. 5.6 is a location map of the eight stations used for sampling at the Homebush site. Fig. 5.7 shows two photographs where *G. holbrooki* only were caught. Fig 5.7A shows the water bird refuge. The substrate at this station was composed of a dense black mud. Sparse plantings of *Acacia* sp. and *A. marina* lined the area. The bank was lined with grass and there were plantings of *Salicornia*. Algal growth was pronounced in the water. There was an extremely strong odour of hydrogen sulphide, evidence of an anaerobic environment. Dried salt lined the edge of the water which was evidence of the very saline conditions.

At the other extreme Fig. 5.7B shows station 8 which was a freshwater pond. The substrate was soft and brown in colour. There was a variety of vegetation lining the pond. On one side *A. marina* was evident, whilst planted eucalypts and *Casuarina* sp. also lined the pond. *Sagittaria* sp. was found in the water. Only *G. holbrooki* was collected. There was an anaerobic odour.

In contrast Fig. 5.8 shows two stations both of which were inhabited by *P. signifer*. Station 6 (Fig. 5.8A) was on the boardwalk on the main channel draining the mangroves. The vegetation was composed only of *A. marina*. The substrate was composed of dark brown mud. Both *P. signifer* and *G. holbrooki* inhabited this station. On the other hand at station 7 (Fig. 5.8B) *P. signifer* only were caught. This station was situated on the main tidal drainage channel of the stand of mangroves.

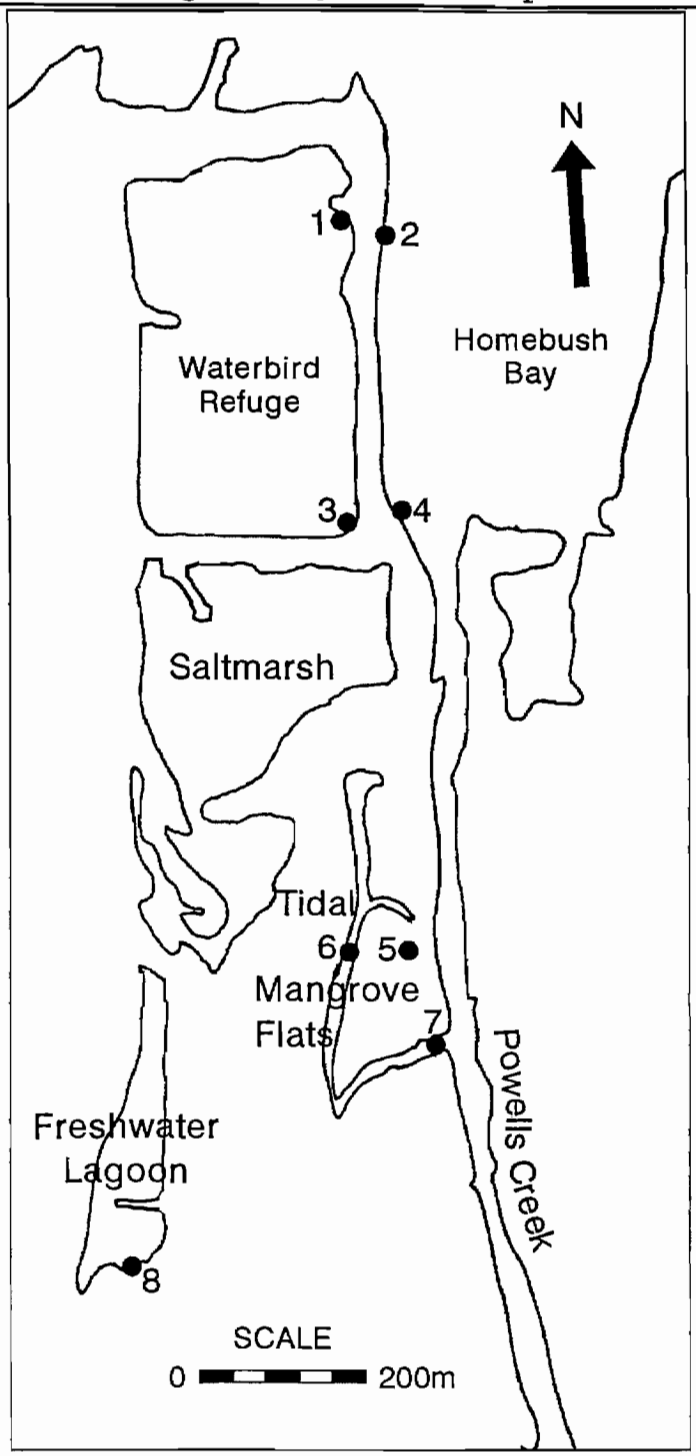


Figure 5.6 Location of the eight stations used for sampling *Pseudomugil signifer* and *Gambusia holbrooki* at Homebush Bay (Site 1 Figure 5.1) in December, 1993.

A

B

Figure 5.7 (A) Station 1 at Homebush Bay a highly saline station where *Gambusia holbrooki* only were caught in December, 1993. (B) Station 8 at Homebush Bay, a freshwater pond where *G. holbrooki* only were caught in December, 1993.

A

B

Figure 5.8 (A) Station 6 on the boardwalk in the main channel draining the mangroves at Homebush Bay where *Pseudomugil signifer* and *Gambusia holbrooki* were caught in December, 1993. The vegetation was strictly *Avicennia marina*. (B) Station 7 at Homebush Bay where *P. signifer* only were caught in December, 1993. The vegetation was *Avicennia marina*.

The vegetation and substrate were similar to that of station 6 but the flow of water was faster.

5.2.2.2 Mooney Mooney Creek, Hawkesbury River.

The sampling site was situated 33° 26'S. 151° 15'E. Mooney Mooney Creek is a north bank tributary of the Hawkesbury River. It is tidal at the sampling site. This area is a section of a river valley that is deeply cut into Hawkesbury sandstone and is part of the Terrigal formation which is composed of shales, laminites and sandstone (Herbert, 1980). The bottom composition is similar to that found at Lane Cove and Narrabeen.

Fig. 5.9 is a location map of the eight stations used for sampling at Mooney Mooney Creek. Fig. 5.10 shows photos of typical vegetation and substrate at the Mooney Mooney Creek Site.

The riparian vegetation and the substrate was relatively consistent between stations. Fig. 5.10A shows typical vegetation and substrate at the Mooney Mooney Creek site. It is a view taken from station 3 on the right bank looking across to the left bank where station 7 was situated. The left side of the creek had sparse mangroves lining the river bank with eucalypts behind them on a steeply inclined bank. The right bank also had mangroves but in addition *Casuarina* sp. were present. The substrate was composed of a light brown sandy mud (Fig. 5.10B) with numerous small rocks on the left side of the creek but on the right side was of a darker colour and more muddy in consistency.

5.2.3 Rainfall.

The Lane Cove and Homebush Bay sites could be considered to have a similar amount of rainfall to that collected at the Sydney Regional Office of the Bureau of Meteorology. Rainfall data for Sydney in 1993 are presented in Table 5.1. The Narrabeen and Mooney Mooney Creek sites could be considered to have similar rainfall to that collected at the Newport Collecting station by the Bureau of Meteorology. 1993 rainfall data collected at Newport is also presented in Table 5.1.

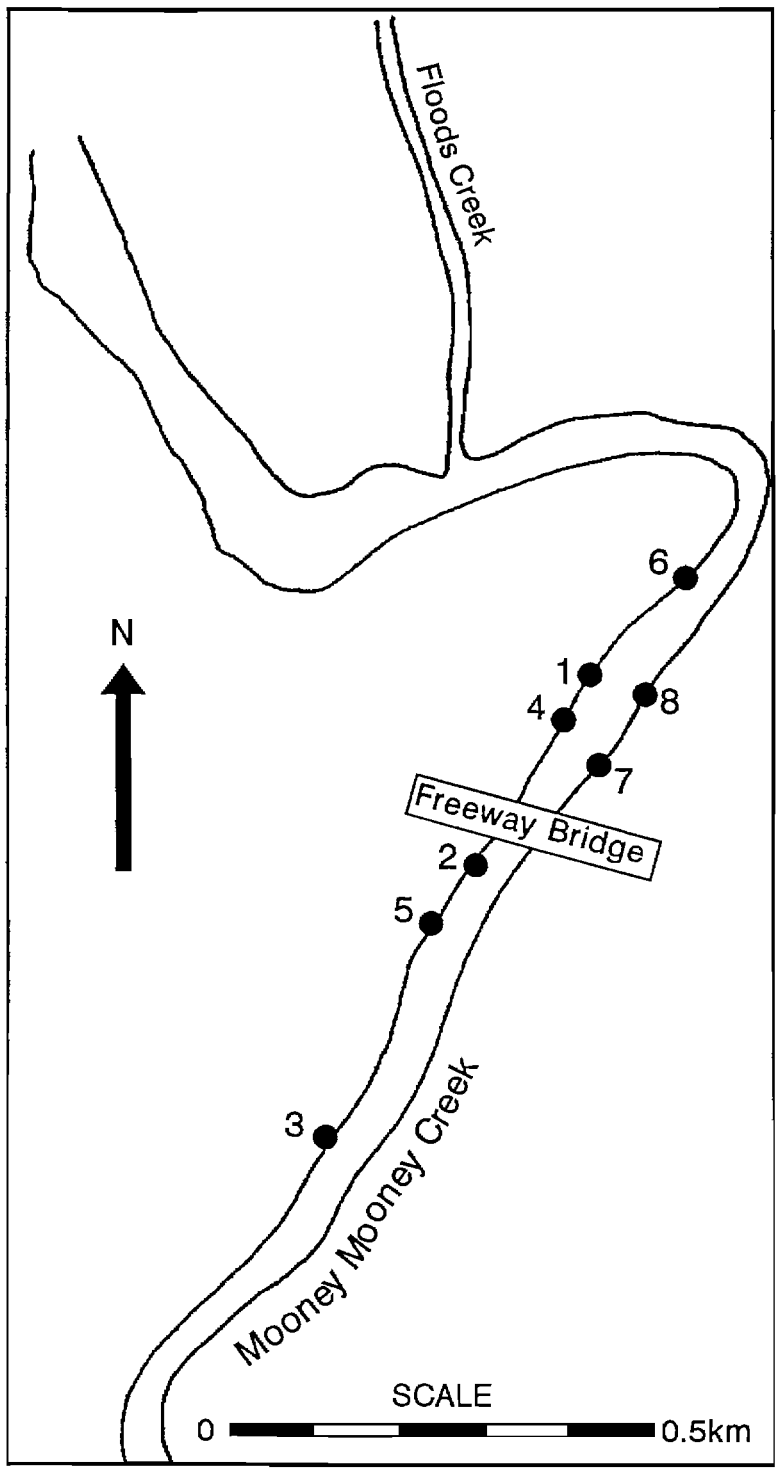


Figure 5.9 Location of the eight stations used for sampling *Pseudomugil signifer* at Mooney Mooney Creek (Site 4, Figure 5.1) in December, 1993

A

B

Figure 5.10 (A) Mooney Mooney Creek sampling site (under Mooney Mooney Bridge) looking towards station 7 in December, 1993. (B) Typical substrate found at Mooney Mooney Creek in December, 1993.

Table 5.1 Rainfall during 1993 at Sydney and Newport, measured by the Bureau of Meteorology.

Rain	Sydney	Newport
Total 1993	913mm	724.8mm
Total average (1858-1993)	1226.8mm	1242.4mm (1931-1993)
Total December, 1993	35.6mm	77.7mm
Total average, December	79.4mm (1858-1993)	81.9mm (1931-1993)
Rainy days, December, 1993	10	9
Average Rainy days, December	12 (1858-1993)	8 (1931-1993)

For the Sydney data the total average rainfall, total average rainfall in December and rain days in December were calculated from data collected between 1858-1993 at the Sydney Regional Office of the Bureau of Meteorology. For the Newport data the total average rainfall, total average rainfall in December and rain days in December were calculated from data collected between 1931-1993 at the Newport Collecting Station (Newport Bowling Club) of the Bureau of Meteorology.

5.2.4 Water quality.

Water quality parameters were determined at each station at each site. Water temperature, pH, dissolved oxygen and salinity were measured on site. Triplicate water samples were taken back to the laboratory where measurements of ammonia and turbidity were made. Means and standard errors of water parameters at each station were calculated.

The pH and temperature were measured with the same instruments as described in Chapter 4. Dissolved oxygen was measured as percentage saturation with an Activon Model 401 Oxygen Meter and converted to mg/l using tables for oxygen solubility corrected for temperature (Activon Model 401 Oxygen Meter Operating Instruction

Manual). Salinity was measured using a YSI Model 33 salinity-conductivity-temperature meter. The units used were g/l. Total ammonia was measured as described in Chapter 4. The un-ionised ammonia was calculated (using measured total ammonia concentrations from the samples) from tables of percent un-ionised ammonia in aqueous ammonia corrected for pH and temperature (Piper *et al.*, 1982). The units used were μg/l. Turbidity is a measure of the optical property that causes light to be scattered and absorbed rather than transmitted directly through a sample (APHA, 1992). It is caused by organic and inorganic particulate matter, water colour and colloidal matter (Oades, 1982). Measurements of turbidity were made using a laboratory turbidity meter Model 2100A, (Hach Chemical Company Iowa). It was measured in Nephelometric Turbidity Units (N.T.U.).

5.2.5 Sampling.

It was determined in a previous study (Howe and Howe, 1991 and Chapter 3) that *P. signifer* had a seasonal reproductive cycle and that they were most reproductively active in the summer months. Sampling was carried out once at each site in December, as previous research (Chapter 3) indicated that the ovary was in peak condition from October to January (class 5 oocytes present) and in a regressed state during winter. The present study was therefore undertaken in order to assess the magnitude of any reproductive impacts (fecundity, ovarian or testis weight change or gonadosomatic index differences) on *P. signifer* due to the presence of *G. holbrooki.*

Temporal variability was reduced by sampling over a short time period in mid December (Table 5.2.).

Table 5.2 Dates of sampling at the four sites.

SITE	DATE SAMPLED
Narrabeen	11th December, 1993
Mooney Mooney Creek	12th December, 1993
Homebush Bay	18th December, 1993
Lane Cove River	19th December, 1993

There were eight field stations at each study site. An equal time of thirty minutes sustained effort was spent at each station collecting fish. The same equipment as described in Chapter 3 was used for sampling. Fish were caught at a maximum depth of ninety centimetres as the two-poled hand net could not be used effectively at any greater depth. The fish could be seen swimming in the shallows and adult fish were actively pursued.

5.2.5.1 Fish measurements and observations.

The fish were brought back live to the laboratory where they were identified, counted, sexed, weighed and measurements of both total and standard lengths were taken. The lengths were measured with a measuring tube (Litvak, 1983). The general condition of *P. signifer* was also assessed by examining the fish for obvious diseases or any sign of emaciation or fin damage (as evidenced by fin nipping).

5.2.5.2 Gonad weight and fecundity.

Gonad weights were measured after the fish were killed using 0.2 g/l of MS222 methane sulphonic acid. The ovaries and testes were dissected out and weighed. The ovaries were then teased apart under a dissecting microscope and the number of large oocytes counted to assess fecundity. Large oocytes were defined as class 5, larger than 0.52mm in diameter, as described in Table 3.1. A graticule was used to determine the diameter of the oocytes. The gonadosomatic index (GSI) was calculated by dividing the gonad weight by the weight of the fish.

5.2.6 Statistical analysis.

5.2.6.1 Water quality.

For all water parameters (pH, temperature, un-ionised ammonia and salinity) except dissolved oxygen and turbidity the Kruskal-Wallis nonparametric test was used as there was a significant difference in standard deviations between sites.

For dissolved oxygen and turbidity a one-way analysis of variance (ANOVA) with Bartlett's test for homogeneity of variances was used.

5.2.6.2 Fish parameters.

Total length, standard length and weight.

The data for females showed a significant difference in standard deviations between sites. Therefore, the Kruskal-Wallis nonparametric test was used on both male and female data.

The observed differences in weights between sites and the variation within sites in this pilot study were used to calculate the power of the analysis, and to predict the extent and nature of sampling necessary to obtain sufficient statistical power to precisely identify differences between sites (Glantz, 1992).

Ovarian weight, testis weight, gonadosomatic index and fecundity of female *P. signifer*.

Kruskal-Wallis nonparametric ANOVA was used as there was a significant difference in standard deviations between sites.

The observed differences between sites and the variation within sites in this pilot study were used to calculate the power of the analysis, and to predict the extent and nature of sampling necessary to obtain sufficient statistical power to precisely identify differences between sites (Glantz, 1992).

Gonadosomatic index of male *P. signifer*.

A one-way analysis of variance (ANOVA) with Bartlett's test for homogeneity of variances was used.

Abundances.

The Kruskal-Wallis nonparametric test was used to compare the abundances between sites as there was a significant difference in standard deviations between sites. Multiple linear regressions were performed on *P. signifer* against all measured water parameters and the presence of *G. holbrooki* at the 32 stations. In addition, the 32 stations were divided into two groups based on the presence or absence of *P. signifer*, independent of site and the data analysed using the nonparametric Mann Whitney test.

The observed differences between sites and the variation within sites in this pilot

study were used to calculate the power of the analysis, and to predict the extent and nature of sampling necessary to obtain sufficient statistical power to precisely identify differences between sites (Glantz, 1992).

5.3 Results.

5.3.1 Rainfall.

The total rainfall at both collection localities (Sydney and Newport) for 1993 was lower than average. Furthermore, the low total rainfall at Sydney in December 1993 appeared to be atypical for this month. Sydney had less than half the total rainfall at Newport in December 1993 (Table 5.1).

5.3.2 Water quality.

The mean water quality results from each site are tabulated in Table 5.3. In addition, the water quality data for the individual stations at the Homebush site are tabulated separately in Table 5.4 as the water quality parameters were variable between stations.

The mean water quality parameters were markedly more variable at the Homebush site than at the other sites (Table 5.3).

Table 5.3 Mean water quality parameters ± standard error except for pH where the range and median are given (field study December, 1993).

Site	Temp°C	pH (range, median)	Salinity g/l	Turbidity (N.T.U.)	Un-ionised NH_3 (μg/l)	D.O. (mg/l)
HB	27±2	7.2-8.9, 7.8	30±4	9.0±2.8	5.07±2.32	4.24±2.00
Nar	27±1	7.2-8.1, 7.9	23±1	8.8±1.8	1.14±0.33	7.89±0.37
L.C.	26±0	7.5-7.9, 7.8	30±0	7.4±1.1	0.83±0.31	8.26±0.21
M.M.	27±0	7.2-7.5, 7.4	26±0	7.5±1.3	0.62±0.04	6.83±0.18

HB - Homebush Bay, Nar- Deep Creek Narrabeen, L.C. - Lane Cove River.
M.M. - Mooney Mooney Creek, Hawkesbury.
Temp °C - Temperature degrees centigrade, D.O. - Dissolved Oxygen.

Table 5.4 Water quality parameters at individual stations at the Homebush site.

Station	Temp °C	pH	Salinity g/l	Turbidity (N.T.U.)	Un-ionised NH_3 (μg/l)	D.O. (mg/l)
1 Gam	32	8.9	39	10	13.28±4.17	-
2 Neither	27	8.1	30	3.5	1.93±0.41	9.2
3 Gam	34	8.9	39	6	17.47±1.78	-
4 Neither	27	8.1	30	8.5	4.12±0.92	9.1
5 Both	21	7.4	36	6.7	0.14±0.084	0.8
6 Both	23	7.4	35	5	0.55±0.06	1.1
7 Both	23	7.2	31	4	1.05±0.14	1.0
8 Gam	30	7.4	1	28	2.05±0.03	-

Key
Gam- *G. holbrooki* only caught. Neither - Neither *P. signifer* or *G. holbrooki* were caught. Both- Both *P. signifer* and *G. holbrooki* were caught.

There were no stations at the Homebush site where *P. signifer* only were caught.

5.3.2.1 Temperature. (Fig. 5.11A).

There were no significant differences between temperature readings at the four sites which averaged between 26°C and 27°C (Table 5.3 and Fig. 5.11). However, the Homebush site had a wider range of temperatures (21-34°C) (Table 5.4) than the other sites (25-29°C). At the Homebush site *G. holbrooki* only were caught at stations with temperatures of 30°C and 32°C (Stations 8 and 1 respectively) in keeping with the range that Otto (1973) reported for *G. holbrooki* (Table 5.4). However, both *P. signifer* and *G. holbrooki* were caught at station 4 at Narrabeen at a temperature of 29°C which was slightly outside the reported range for *P. signifer* by Allen and Cross (1982).

5.3.2.2 pH. (Fig. 5.11B).

The median pH value for the Narrabeen site (7.85) was found to be significantly higher than that of the Mooney Mooney Creek site (7.40) ($P<0.05$ Kruskal-Wallis nonparametric ANOVA with Dunn's post-tests). No other differences were found

between sites. The pH levels of the four sites fell within the range in which *P. signifer* has been reported to occur (Allen and Cross, 1982).

5.3.2.3 Salinity. (Fig. 5.11C).

Full strength seawater is approximately 30-40 g/l (Ferguson, 1988). The salinity at Narrabeen (mean of 23 g/l) was significantly lower than at Homebush (mean of 30 g/l) and at Lane Cove (mean of 30 g/l). The salinity at Mooney Mooney Creek (mean of 26 g/l) was not significantly different from any of the other sites. It should be noted that at the Homebush site there were relatively large variations in salinity between stations (Station 8 had a salinity of 1 g/l, whilst Stations 1 and 3 had a salinity of 39 g/l all of which had *G. holbrooki*). *P. signifer* are found in marine waters through to freshwater (Allen and Cross, 1982).

Gee (1988) has successfully transferred *P. signifer* from 5 g/l to 35 g/l. Sicault (1934), cited in Krumholz (1948) found *G. affinis* could survive in 33 g/l sodium chloride in water but died at concentrations greater than 35 g/l.

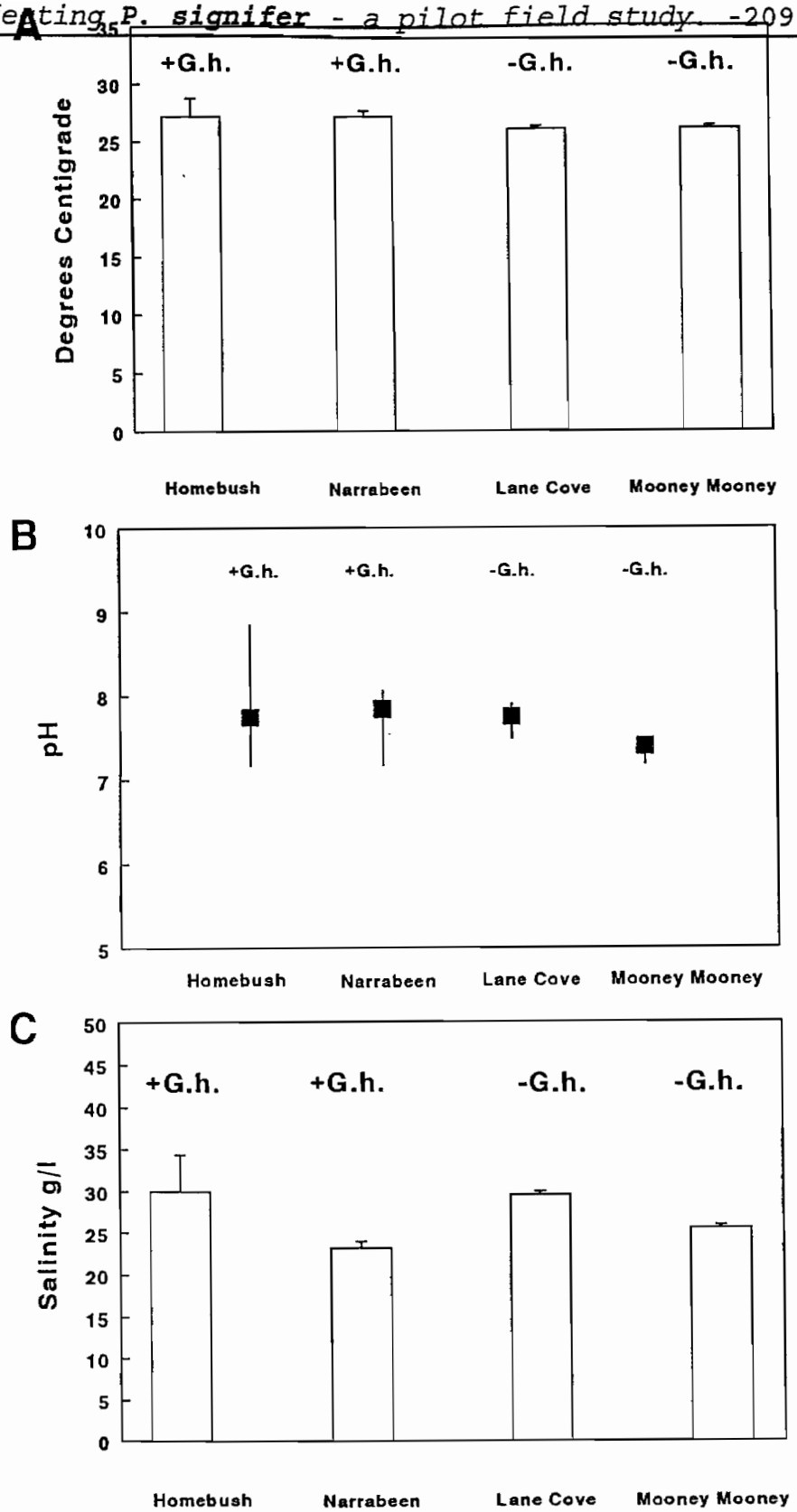


Figure 5.11 Temperature (Mean ± SE) (A), pH (median and range) (B) and salinity (Mean ± SE) (C) at the four sampling sites (Homebush Bay, Narrabeen, Lane Cove and Mooney Mooney) used in December, 1993.
+G.h. indicates Gambusia holbrooki present at the site with *Pseudomugil signifer*
-G.h. indicates *G.* holbrooki not present.

5.3.2.4 Turbidity. (Fig. 5.12A).

Recommended levels for total suspended solids in freshwater aquaculture are 0.03mg/l (Cook, 1986). However, a poor correlation has generally been reported between turbidity and total suspended solids (APHA, 1992; Oades, 1982; Webb and Walling, 1992). It is difficult to know whether levels found in this study are acceptable. Earlier literature on water quality criteria does not contain discussions of this parameter (Hart, 1974; EPA, 1976) and there is still no clear agreement on acceptable standards for aquatic ecosystems (ANZECC, 1992). Tentative acceptable concentrations for finely dissolved solids (Alabaster and Lloyd, 1982) were not based on data for Australian species.

The Narrabeen site had the lowest turbidity with a mean of 5.8 N.T.U. and the Homebush site the highest with an average of 9.0 N.T.U. The Homebush site had the widest range of turbidities (2.3-15.6 N.T.U.) compared to a maximum level of less than 11 at the other sites. However, there was no significant difference in turbidity between sites.

5.3.2.5 Un-ionised ammonia (Fig. 5.12B).

The concentrations of un-ionised ammonia at the four sites studied were acceptable based on the criteria of Redacliff (1985) and Cook (1986). There was no significant difference in the mean values between sites. The Homebush site had the largest range (0.14-17.47 μg/l) in concentration of un-ionised ammonia.

The maximum level of un-ionised ammonia at the other sites was less than 3 μg/l. The literature does not specify the tolerances of either *P. signifer* or *G. holbrooki* to un-ionised ammonia.

5.3.2.6 Dissolved oxygen (Fig. 5.12C).

G. holbrooki and *G. affinis* can tolerate extremely low levels of oxygen (Sjogren, 1972 and Cech *et al.*, 1985).

No data for dissolved oxygen requirements in *P. signifer* could be found in the literature but Alabaster and Lloyd (1982) found that mature fish can probably survive in dissolved oxygen levels in excess of 3 mg/l when other conditions are favourable.

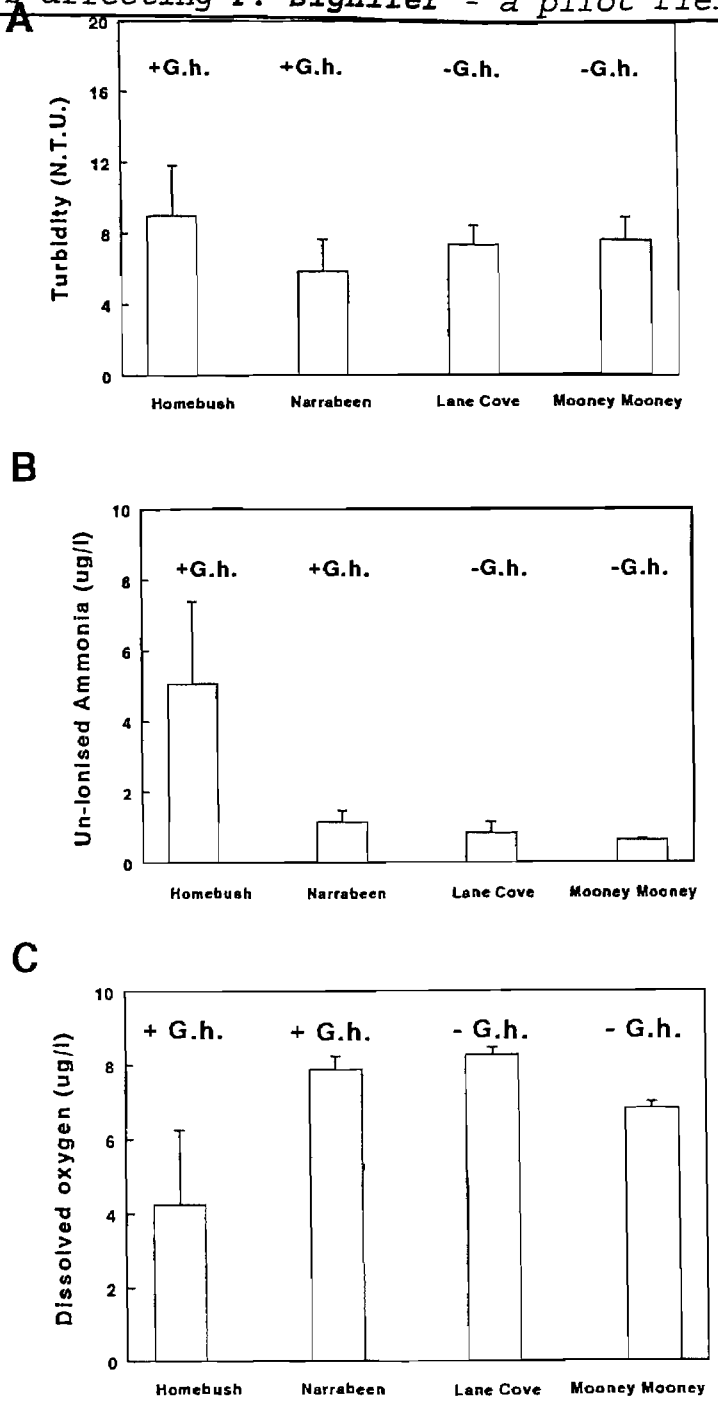


Figure 5.12 Turbidity (A), un-ionised ammonia (B) and dissolved oxygen (C) at the four sampling sites (Homebush Bay, Lane Cove, Narrabeen and Mooney Mooney) used in December, 1993 (Mean ± SE).
+G.h. indicates *Gambusia holbrooki* present at the site with *Pseudomugil signifer* - G.h. indicates *G. holbrooki* not present.

The mean dissolved oxygen concentration at the Homebush site (4.24 mg/l) was significantly lower than both the Lane Cove (8.26 mg/l) and Narrabeen (7.88 mg/l) site ($P<0.01$, $P<0.05$ respectively, one-way ANOVA).

5.3.3 Measurements of fish.

5.3.3.1 Abundances (Fig. 5.13).

The mean number (males and females) of *P. signifer* was significantly higher at Mooney Mooney Creek than at Homebush Bay (Fig. 5.13B) ($P<0.05$ Kruskal-wallis non-parametric ANOVA with Dunn's post-test). In addition the mean number (males and females) was significantly higher at Mooney Mooney Creek than at Lane Cove ($P<0.05$ Kruskal-Wallis non-parametric ANOVA with Dunn's post-test). It was observed that the *Zostera capricornia* beds where *P. signifer* had previously been found to be abundant at Deep Creek, Narrabeen (Chapter 3) were nearly absent.

There appeared to be more *P. signifer* at the sites where *G. holbrooki* were absent (Lane Cove and Mooney Mooney Creek) (Fig. 5.13A) although the difference was found not to be significant. Given the small number of sites sampled, the power of the analysis was found to be too low (<0.35) to draw any firm conclusions. To obtain sufficient power ($\alpha=0.05$, $\beta=0.05$) to assess differences between abundances at sites with and without *G. holbrooki* would require at least 25 samples to be taken at each site in the case of both males and females, with a comparison of the Lane Cove and Homebush sites requiring more than 100 such samplings. Follow-up, more extensive sampling was beyond the scope of this project though the pilot study has been useful in indicating requirements needed to establish, unequivocally the interrelationships of the two species.

Variability of the Homebush site - summary.

There were significantly more *G. holbrooki* at Homebush (71 total) than at Narrabeen (15 total). At Homebush there were 2 stations with neither species, 3 with only *G. holbrooki* and 3 with both.

At the stations where *G. holbrooki* only were caught two had very high salinity, temperature and pH values (Station 1 and 3) and one had a very low salinity, with high turbidity and temperature values (Station 8). *G. holbrooki* were caught at the

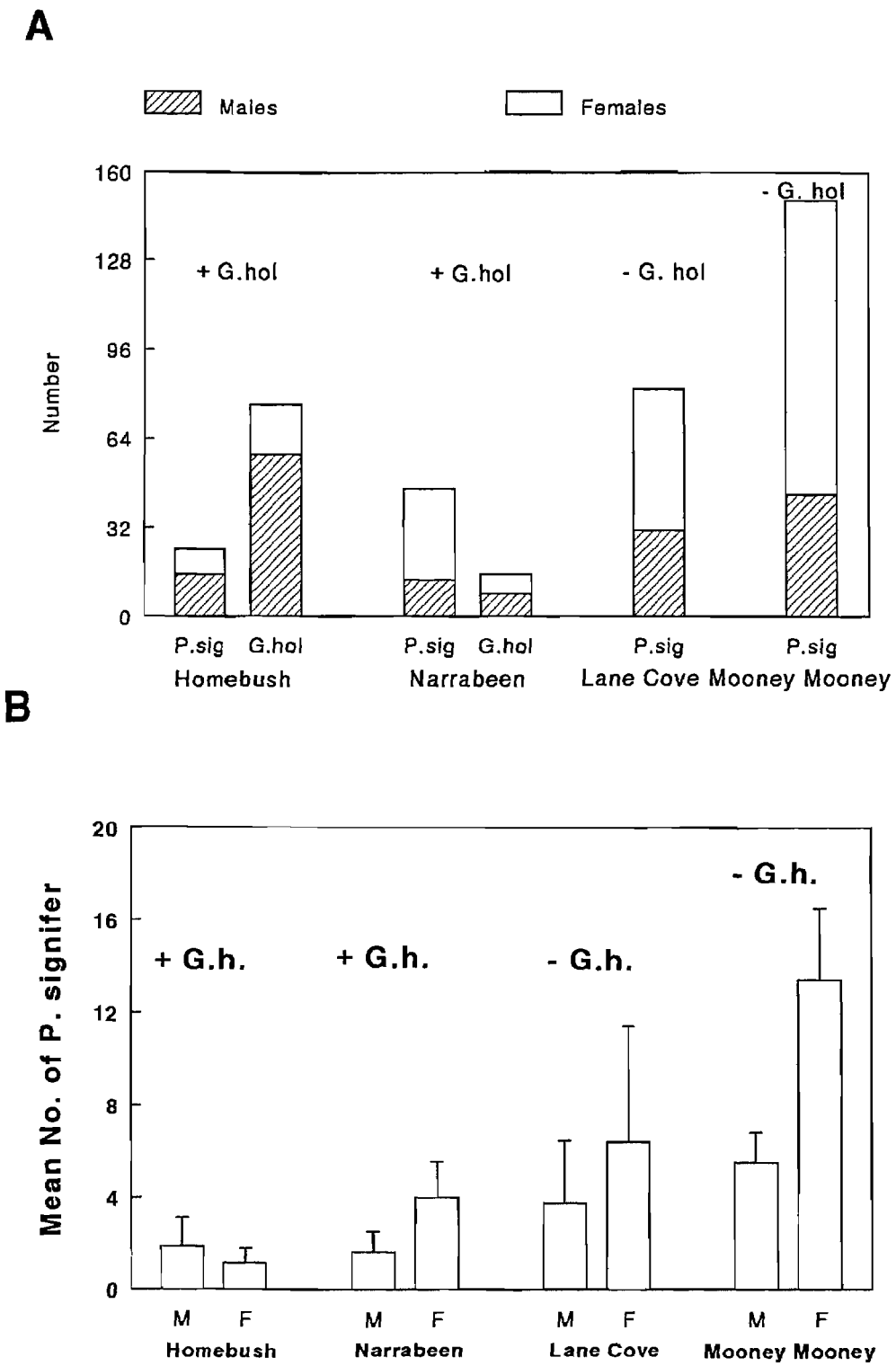


Figure 5.13. The total number of male and female *Pseudomugil signifer* and *Gambusia holbrooki* (A) and the average number of *P. signifer* male (M) and female (F) (± SE) (B) caught at the sampling sites (Homebush Bay, Narrabeen, Lane Cove and Mooney Mooney) in December, 1993.
+G.h. indicates *G. holbrooki* present at the site with *P. signifer* and
-G.h. indicates *G. holbrooki* not present.

two extremes of salinity. At the three stations where both species were caught (Stations 5, 6 and 7), there was a relatively low turbidity, a near neutral pH and un-ionised ammonia concentrations that were lower (<1.05 μg/l) than other stations.

Examination of abundance by multiple regression analysis.

The abundance of *P. signifer* was positively correlated with turbidity (r= 0.37, P= 0.017). Although the relationship exists it is probably a weak one. The abundance of *P. signifer* was not correlated with any other water quality parameter. The abundance of *G. holbrooki* positively correlated with un-ionised ammonia (r= 0.94, P= 0.02). There was also a suggestion of a negative correlation of male *P. signifer* with un-ionised ammonia (r= 0.94, P=0.06).

Stations separated on the basis of presence or absence of *P. signifer* and compared with water quality parameters and the presence of *G. holbrooki*.

P. signifer were present at 22 out of the 32 stations. *P. signifer* were absent from 5 stations at Homebush Bay, 0 at Mooney Mooney Creek, 1 at Narrabeen and 4 at Lane Cove. The pH (P=0.004, Mann-Whitney test), temperature (P=0.030, Mann-Whitney test) and dissolved oxygen (P=0.001, Mann-Whitney test) were significantly lower in those stations where *P. signifer* were caught. The salinity tended to be lower (P=0.084, Mann-Whitney test).

5.3.3.2 Total and standard length and weight (Fig. 5.14 A, B and C).

Females of *P. signifer* from Narrabeen were significantly longer and heavier than females from either the Lane Cove (P<0.001 (TL), P<0.05 (Wt); Kruskal-Wallis nonparametric ANOVA with Dunn's post-test) or Mooney Mooney (P<0.001 (TL), P<0.01 (Wt); Kruskal-Wallis nonparametric ANOVA with Dunn's post-test) sites.

The weight of male *P. signifer* at Narrabeen was significantly lower than at Lane Cove (P<0.05; one way ANOVA with Tukey-Kramer post test) (Fig 5.14C). Again, the ability to discriminate between the groups was limited by the low power of the analysis (<0.35 in all cases). The number of fish required to obtain satisfactory statistical power (83 to 1,879, depending on site) was generally several times the number actually analysed. It was calculated from the numbers of fish

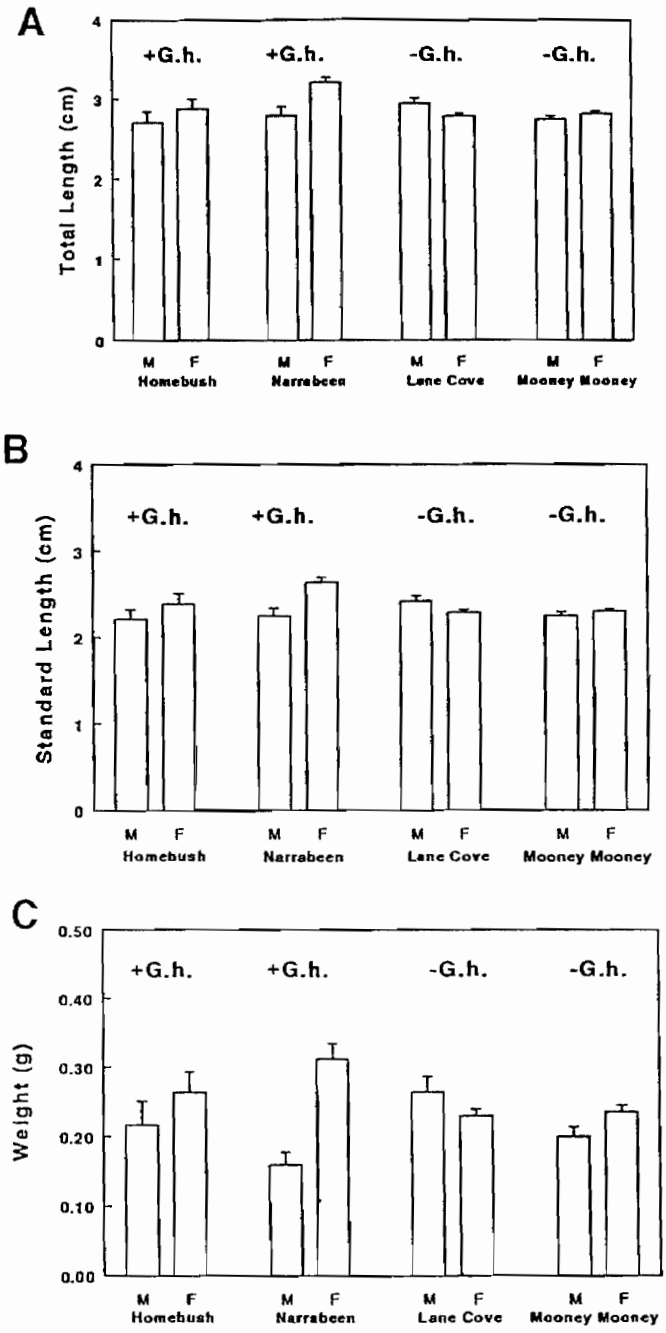


Figure 5.14 The total lengths (A), standard lengths (B) and weights (C) of male (M) and female (F) *Pseudomugil signifer* at the four sampling sites (Homebush Bay, Narrabeen, Lane Cove and Mooney Mooney) in December, 1993 (± SE).
+G.h. indicates *Gambusia holbrooki* present at the site with *P. signifer* and -G.h. indicates *G. holbrooki* not present.

obtained in these sampling trips, that between 14 and 20 trips to each site, each sampling at eight stations, would be required to discriminate between sites thought likely to differ. Such an experimental regime, however would also involve the confounding effects of variations in the environmental conditions of the sites.

5.3.3.3 Testis weight (Fig. 5.15A).

Males of *P. signifer* from Homebush had significantly heavier testes than males from either the Narrabeen ($P<0.05$, Kruskal-Wallis nonparametric ANOVA with Dunn's post-test) or Mooney Mooney Creek ($P<0.001$, Kruskal-Wallis nonparametric ANOVA with Dunn's post-test) sites.

Male *P. signifer* from Lane Cove had significantly heavier testes than those from the Mooney Mooney Creek site ($P<0.01$, Kruskal-Wallis nonparametric ANOVA with Dunn's post-test) (Fig. 5.15A). No other significant differences were observed.

5.3.3.4 Ovarian weight.

No significant differences were found among sites in ovarian weight of *P. signifer* (Fig. 5.15B). Again the very low power of the analysis (<0.35) raises the considerable likelihood of a Type II error ($\beta>0.65$) in assessing the ovarian weights of the fish at the various sites. The number of fish to be sampled to yield adequate statistical power, given the differences between means and the variation within sites, was very large, for example, several thousand fish would be required to confidently determine whether any difference exists between ovarian weights in fish from Homebush and Mooney Mooney. Other salient comparisons would require hundreds of specimens. Given the number of fish obtained at each sampling in the present pilot study, a very large sampling effort would be required. Ovarian weight was plotted against body weight (Fig. 5.16). At all sites, there were significant correlations between ovarian weight and body weight (see Fig. 5.16 for details). The slopes of the linear regression lines were compared using ANOVA with the Tukey-Kramer post-hoc test. The slope of the regression line for the Lane Cove fish was significantly less than that for the Mooney Mooney fish ($P<0.001$).

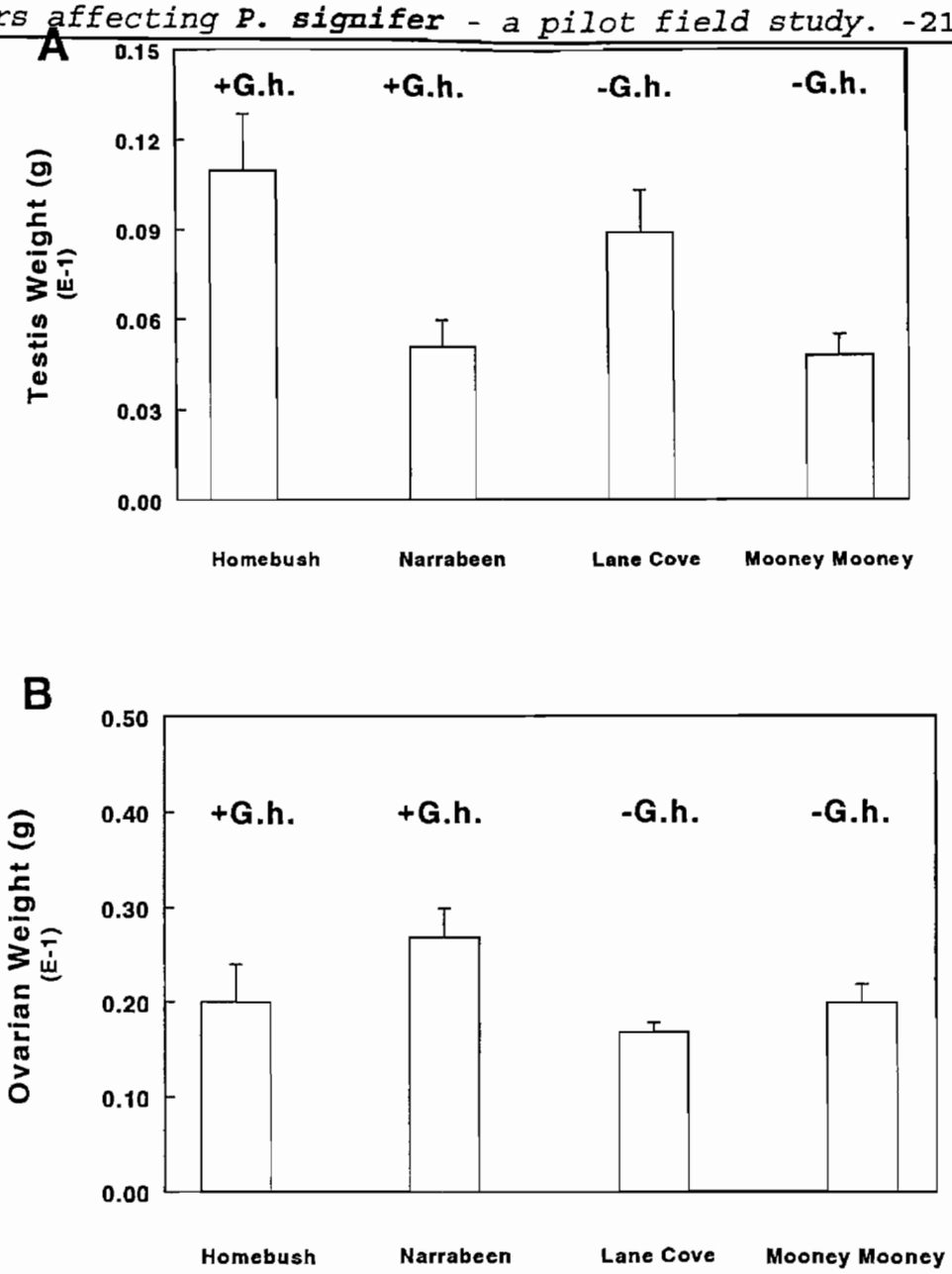


Figure 5.15 The average testis weight (A) and ovarian weight (B) of *Pseudomugil signifer* at the four sampling sites (Homebush Bay, Narrabeen, Lane Cove and Mooney Mooney) in December, 1993 (± SE).
+G.h. indicates *Gambusia holbrooki* present at the site with *P. signifer*.
-G.h. indicates *G. holbrooki* not present.

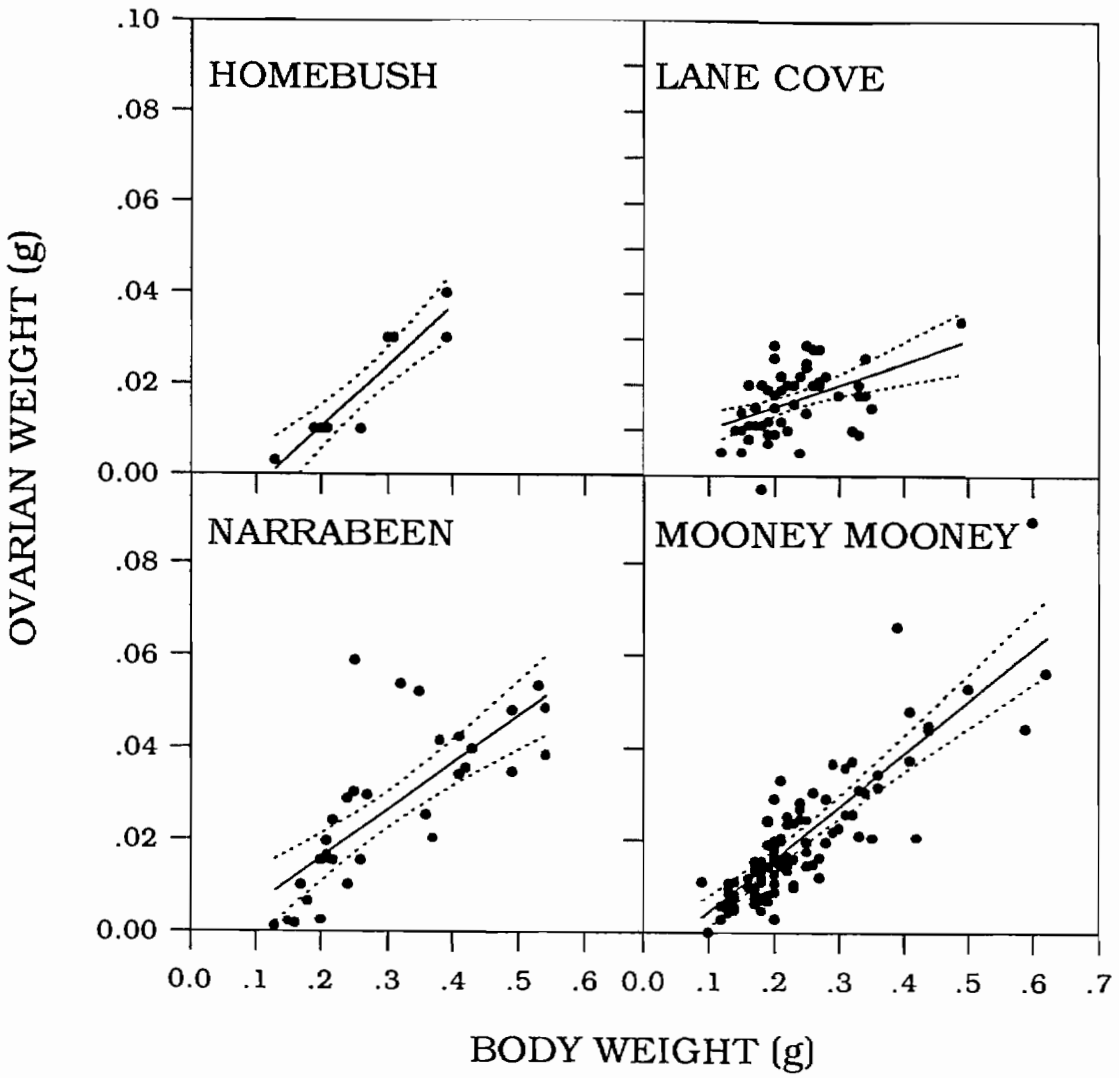


Figure 5.16 Ovarian weight plotted against body weight at harvest in all four sites. There were significant correlations between ovarian weight and body weight at all sites. Scales on all graphs are equal to permit comparison.

A: Homebush, n=9.
Significant positive correlation between ovarian weight and body weight ($P = 0.0003$).

B: Lane Cove, n=51.
Significant positive correlation between ovarian weight and body weight ($P = 0.0003$).

C: Narrabeen, n=33.
Significant positive correlation between ovarian weight and body weight ($P < 0.0001$).

D: Mooney Mooney, n=107.
Significant positive correlation between ovarian weight and body weight ($P < 0.0001$).

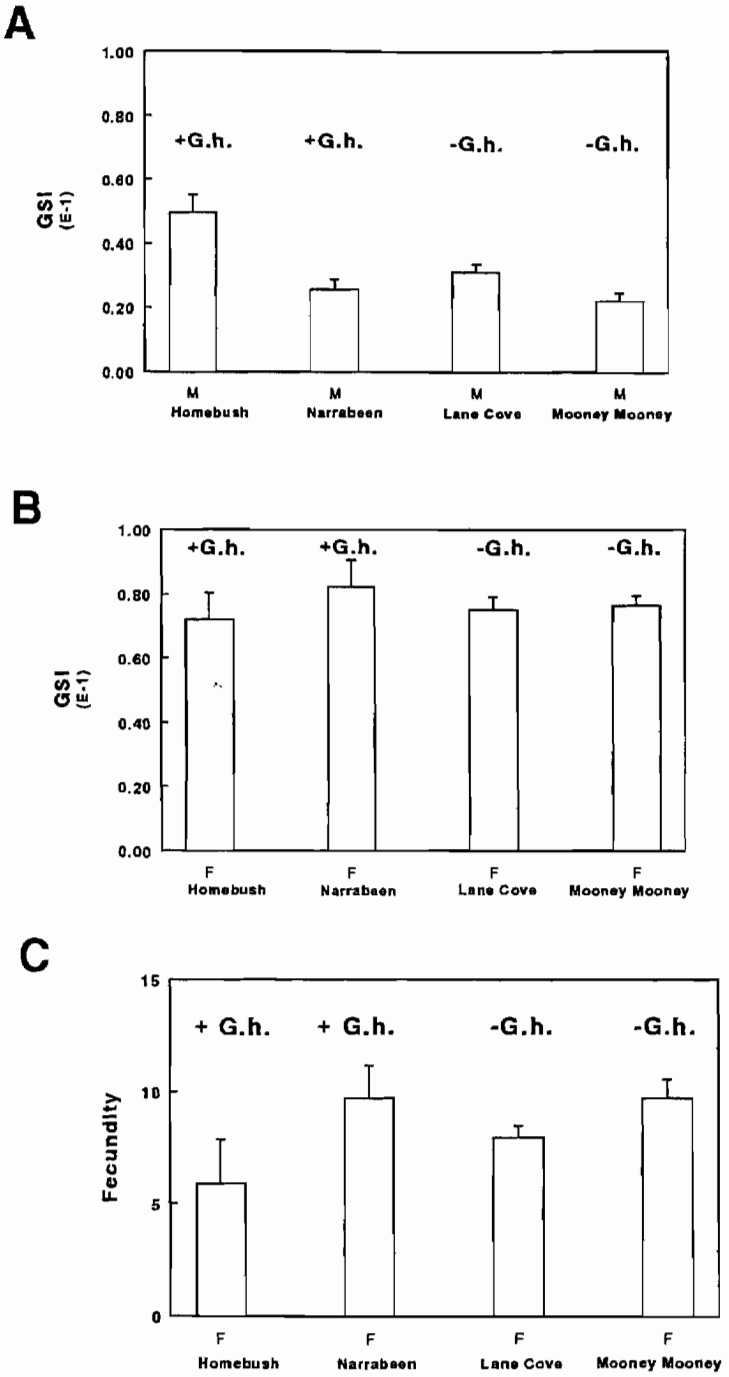


Figure 5.17 Average gonadosomatic index (GSI) ± SE of male (A), female (B) and average fecundity ± SE (C) of *Pseudomugil signifer* at the four sampling sites (Homebush Bay, Narrabeen, Lane Cove and Mooney Mooney) in December, 1993. +G.h. indicates *Gambusia holbrooki* present at the site with *P. signifer*. -G.h. indicates *G. holbrooki* not present.

5.3.3.5 Gonadosomatic index (GSI) of male *P. signifer*.

The GSI of male *P. signifer* from Homebush Bay were significantly larger than those from Lane Cove ($P<0.001$), Narrabeen ($P<0.001$) and Mooney Mooney Creek ($P<0.001$, one way ANOVA with Tukey's post test) (Fig. 5.17A).

The GSI highlights the findings that male *P. signifer* from the Homebush site have larger testes in relation to their body weight. The GSI of male *P. signifer* from the Narrabeen site did not differ from those at Lane Cove and Mooney Mooney even though males from Narrabeen were smaller than those at Lane Cove and Mooney Mooney.

5.3.3.6 Gonadosomatic index and fecundity of female *P. signifer* (Fig. 5.17 B and C).

No significant differences in gonadosomatic index or fecundity were shown between sites in female *P. signifer*.

No significant differences (one way ANOVA) were found in fecundity between these four sites that were sampled in December, 1993 and those sampled at Lane Cove and Narrabeen in January 1987 (Chapter 3).

Again the very low power of the analysis (<0.35) raises the considerable likelihood of a Type II error ($\beta>0.65$) in assessing the fecundity of the fish at the various sites. The number of fish to be sampled to yield adequate statistical power, given the differences between means and the variation within sites, was about 190 for the sites thought possibly to differ. This represents between three and twenty times as many sampling trips as were conducted in the present study, in order to obtain sufficient data.

Fecundity was plotted against body weight (Fig. 5.18). As with ovarian weight, there was a positive correlation between fecundity and body weight at all sites (see Fig. 5.18 for details). The slopes of the linear regression lines obtained were compared by ANOVA with Tukey-Kramer's post-hoc test. As with ovarian weight the regression line for Lane Cove had a lesser slope than Mooney Mooney ($P<0.01$).

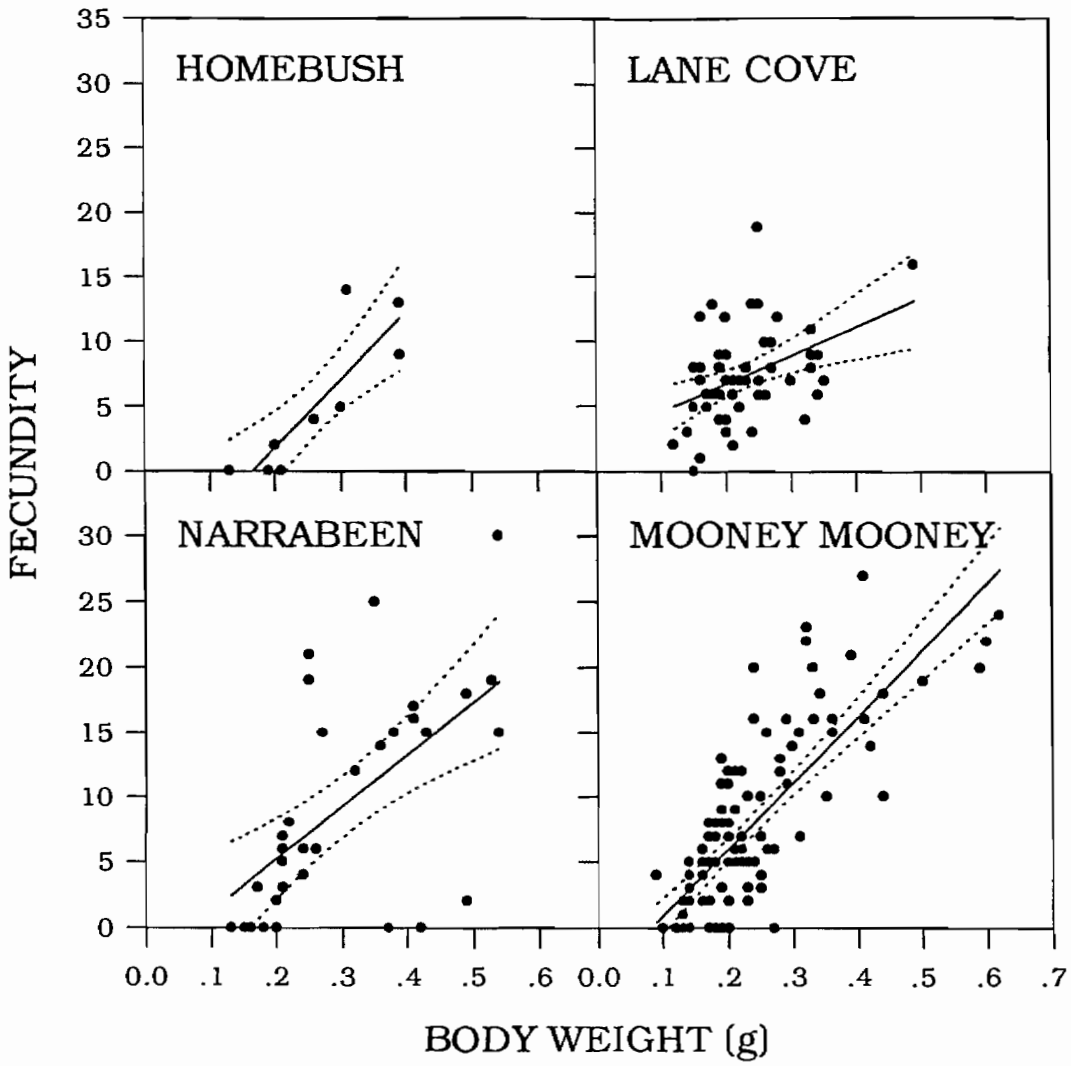


Figure 5.18 Fecundity plotted against body weight at harvest in all four sites. There were significant correlations between fecundity and body weight at all sites. Scales on all graphs are equal to permit comparison.

A: Homebush, n=9.
Significant positive correlation between fecundity and body weight (P = 0.0033).

B: Lane Cove, n=51.
Significant positive correlation between fecundity and body weight (P = 0.0022).

C: Narrabeen, n=33.
Significant positive correlation between fecundity and body weight (P = 0.0002).

D: Mooney Mooney, n=107.
Significant positive correlation between fecundity and body weight (P < 0.0001).

5.3.3.7 Comparison of fecundity in tank experiment II (Chapter 4) and the field study.

The fecundities of *P. signifer* in the tanks where *G. holbrooki* were present, were significantly lower than at any of the field sites apart from Homebush Bay where both species were present (fed and unfed, Lane Cove and Narrabeen $P<0.05$; fed, Mooney Mooney $P<0.01$ and unfed Mooney Mooney $P<0.001$; Kruskal-Wallis nonparametric ANOVA with Dunn's multiple comparisons test). It is difficult to ascertain whether at this site the results could be attributed to the presence of *G. holbrooki* or to water conditions.

At all four sites in the field study there were significant relationships between fecundity or ovarian weight, and body weight. In the tank experiments, such a relationship was observed only in the control treatment with no supplementary feeding. The supplementary fed control group had substantial ovarian weight and fecundity, but it did not relate significantly to body weight. The experimental groups (those with *P. signifer* and *G. holbrooki*) had very low ovarian weights and fecundities across all sizes of *P. signifer*.

A summary of significant analyses of both water quality and fish parameters is presented in Table 5.5.

5.3.4 Caudal fin.

There was no evidence of fin nipping (caudal fin damage) in *P. signifer* at any sites from which they were collected. This was in contrast to the results in the previous chapter where the caudal fin of *P. signifer* housed with *G. holbrooki* showed evidence of fin nipping.

Table 5.5 Summary of significant analyses.

Parameter	Finding	Test	Significance
pH	Narrabeen> Mooney Mooney	Kruskal-Wallis	P <0.01
Salinity	Narrabeen < Homebush & Lane Cove	Kruskal-Wallis	P <0.01
Dissolved Oxygen	Homebush < Narrabeen Homebush < Lane Cove	1-Way ANOVA	P <0.0.05 P <0.01
Abundance *P. signifer*	Total Mooney Mooney > Homebush Total Mooney Mooney > Lane Cove Males Mooney Mooney > Homebush Males Mooney Mooney > Lane Cove Females Mooney Mooney > Homebush	Kruskal-Wallis	P < 0.05 P <0.05 P <0.05 P < 0.05 P <0.01
Abundance *P. signifer*	Correlated with turbidity	Multiple Regression Analysis	P=0.0165
P. signifer present	pH lower Temperature lower Dissolved Oxygen lower	Mann-Whitney	P= 0.0042 P=0.0296 P=0.0008
TL & SL *P. signifer*	Female Narrabeen> Lane Cove Female Narrabeen > Mooney Mooney	Kruskal-Wallis	P< 0.001 P <0.001
Weight *P. signifer*	Male Narrabeen < Lane Cove Female Narrabeen > Lane Cove Female Narrabeen > Mooney Mooney	Kruskal-Wallis	P <0.01 P <0.01 P <0.01
Testis Weight *P. signifer*	Homebush > Narrabeen Homebush > Mooney Mooney Lane Cove > Mooney Mooney	Kruskal-Wallis	P <0.05 P <0.001 P<0.05
GSI *P. signifer*	Males Homebush > Lane Cove Males Homebush > Narrabeen Males Homebush > Mooney Mooney	1-Way ANOVA	P <0.01 P <0.001 P <0.001

5.4 Discussion.

This pilot study was intended to follow on from the twelve month field study that was reported in Chapter 3. The fecundity measurements from the pilot study were not significantly different from those measured in January 1986 but were significantly lower than those of October 1985 indicating that at the time of sampling egg production was just beyond its peak.

In addition the results obtained from the pilot study differ markedly from those found in the tank experiment in Chapter 4. No reduction in lengths, weights, GSI,

fecundity or gonad weight could be directly attributed to the sites where both *P. signifer* and *G. holbrooki* were present. More sampling is required to clarify this point. No caudal fin nipping was found in *P. signifer* that co-habited with *G. holbrooki* in the field. The reasons for such effects as caudal fin nipping being observed only in the tank study could be related to the confined conditions in the studies discussed in Chapter 4. It is plausible that when the two species are kept in a restricted space such as in the tank experiment the effects would be different to that in the field because of crowding, worsening competition or aggression.

In the pilot field study, a linear relationship was found at all sites between ovarian weight and body weight and between fecundity and body weight. It was similar at all sites, with Lane Cove showing a tendency to have a flatter curve in both fecundity and ovarian weight. In the tank studies, a significant relationship between ovarian weight and body weight and between fecundity and body weight occurred only in the control group which received no supplementary feeding. In the experimental groups, where *G. holbrooki* was present, ovarian activity was obliterated. Interestingly, in the control groups which received supplementary feeding, there were substantial fecundity and ovarian weight, which were not correlated to body weight. Although numbers are too small to state this with certainty, this effect appears to have been caused by a slight increase in ovarian weight and fecundity in smaller fish, suggesting that the smaller fish were open to benefit from supplementary feeding. This aspect could be profitably studied further.

From these data, it seems that the tank treatment most like the field sites in terms of ovarian activity was the control group receiving no supplementary feeding.

There are difficulties in sampling in the field that cannot always be taken into consideration when planning a study, hence the necessity for the type of pilot study included here. For example there are difficulties in netting fish which are mobile targets, in differing substrates and underwater snags. Thus laboratory tank studies can be useful in providing uniform conditions and more reliable and reproducible sampling of the fish. The field data do not negate the hypothesis that *G. holbrooki* do have a negative impact on *P. signifer*. A more comprehensive study over several months at more sites would perhaps draw out these differences. This pilot study appears to indicate that water parameters play a more important role in the

abundance of *P. signifer* than the presence of *G. holbrooki*.

As in the study reported in Chapter 3, considerable effort was made to equalise sampling effort by using the same operator, sampling for the same length of time and in the same place at each site. As before, the sampling method used did not allow the collection of large numbers of fish. It did allow sampling in the difficult areas encountered at all four sites.

This was a preliminary study devised to determine the amount of sampling required for a much larger field study on the impact of *G. holbrooki* and physical and chemical factors on *P. signifer*. It was anticipated that the field study to follow this pilot study would be conducted over several seasons.

With the exception of the Narrabeen site (which had significantly longer and heavier female *P. signifer* than either the Lane Cove or Mooney Mooney sites and lighter male *P. signifer* than Lane Cove) the fecundity and size of *P. signifer* did not appear to be affected by habitat conditions between sites (specifically salinity, turbidity and temperature were all much the same) or by the presence of *G. holbrooki*.

It would be useful to perform more extensive spatial and temporal field studies, sampling for abundances, as there was some indication that there were reduced total abundance of *P. signifer* at sites (Deep Creek and Homebush Bay) where *P. signifer* and *G. holbrooki* were present. There were significantly fewer numbers of *P. signifer* at Homebush Bay when compared to Mooney Mooney Creek which could be related to both environmental conditions and the presence of *G. holbrooki*. *P. signifer* were found at stations with lower pH, temperature and dissolved oxygen.

The water quality results are discussed below with respect to their implications for habitat conditions for *P. signifer* and *G. holbrooki*. Water quality factors are also related to specific fish parameters, and the presence or absence of *G. holbrooki*.

5.4.1 Water quality.

5.4.1.1 Temperature.

According to the literature no individual environmental factor affects the growth of fish as much as water temperature (see, eg Piper *et al.*, 1982; Ferguson, 1988). The more restricted range of temperature preferences of *P. signifer* compared to *G.*

holbrooki has been noted before. The results of this study would appear to substantiate this finding in that *G. holbrooki* alone were caught at three stations with a temperature of 30°C or above at Homebush. *P. signifer* were caught at Narrabeen (29°C) at a temperature slightly outside that reported in the literature (15-28°C) (Allen and Cross, 1982) which suggests that ecological data on *P. signifer* should be revised. *G. holbrooki* were able to inhabit waters that were not available to *P. signifer* because of temperature constraints. The differences found in fish size (female *P. signifer* Narrabeen), abundance (Mooney Mooney had larger numbers than Homebush or Lane Cove), and the larger gonadosomatic index (male *P. signifer* from Homebush) could not be directly attributed to temperature as the temperatures at the four sites sampled were not significantly different from one another. However, it was found that the presence of *P. signifer,* when analysed by station, was associated with lower temperatures ($P = 0.030$, Mann-Whitney test).

Temperature is of importance for the breeding cycle of *P. signifer*. In this study *P. signifer* were found to breed seasonally (Chapter 3), commencing breeding as the daylength and temperature increased and declining in breeding activity as the daylength and temperature declined. The seasonality of the reproductive cycle of *G. affinis* is also principally related to photoperiod and temperature (Brown and Fox, 1966; Sawara, 1974 and Davis, 1978).

5.4.1.2 pH.

The slight difference between the median pH at Mooney Mooney Creek (7.40) and that at the Narrabeen site (7.85) is probably of little consequence and could be a result of slight differences in water chemistry. On its own it would be unlikely to have any ecological significance for the fish inhabiting the waters as these levels are well within the acceptable range (6.5-9.0) for fish (Cook, 1986; Alabaster and Lloyd, 1982). However, *P. signifer* were found at the two sites which had a more restricted pH range (less than 8.0). It is not clear why this should be so beyond the obvious observation that *Gambusia* have a broader range of pH tolerance. In addition, the presence of *P. signifer* when analysed by station was associated with a lower pH ($P = 0.004$, Mann-Whitney test).

5.4.1.3 Salinity.

Salinity variations in the Homebush Bay site were large between stations (Station 8 had a salinity of 1 g/l, whilst Stations 1 and 3 had a salinity of 39 g/l. *G. holbrooki* were present at all 3 stations. *P. signifer* are found in marine waters through to freshwater (Allen and Cross, 1982). *P. signifer* are known to be euryhaline (Merrick and Schmida, 1984 and Gee 1988). It is difficult to know, however, whether a salinity of 39 g/l is outside of *P. signifer*'s range as no tests have been carried out at such a high value. The salinity at Narrabeen was significantly lower than that at Homebush and Lane Cove. On a site basis it is difficult to form any conclusions on these differences although female *P. signifer* were found to be longer (both total and standard length) and heavier at Narrabeen. The preference of *P. signifer* for areas with varying salinity is not clearly established even though the tolerance is. Female *P. signifer* could be larger at Narrabeen because *G. holbrooki* ate younger, smaller *P. signifer* leaving the larger ones. A similar effect has been observed in *Poeciliopsis occidentalis sonoriensis* which, when syntopic with *G. affinis* had higher fecundity than *P. o. sonoriensis* in the absence of *G. affinis* (Galat and Robertson, 1992). These authors suggested that the increased fecundity was a result of a larger average size of *P. o. sonoriensis* with *G. affinis*, due to predation by the latter on smaller *P. o. sonoriensis*.

5.4.1.4 Turbidity.

The levels found in this study were not significantly different between sites. However, using multiple linear regression analysis on the 32 stations the abundance of *P. signifer* was positively related to turbidity levels. Turbidity is caused by organic and inorganic matter, water colour and colloidal matter (Oades,1982). If the higher turbidity level is caused by organic matter it could be providing more food for *P. signifer* in the form of zooplankton. In addition the higher levels of turbidity could be offering cover for *P. signifer* against predation.

5.4.1.5 Dissolved oxygen.

It was found that the presence of *P. signifer*, when analysed by stations, was related to lower dissolved oxygen levels ($P = 0.001$, Mann-Whitney test). *P.*

signifer are tolerant of low dissolved oxygen (this was shown in Chapter 4). They are an estuarine species found in mangroves and as such would have to tolerate changes in dissolved oxygen. No data are specifically available in the literature on recommended dissolved oxygen requirements for *P. signifer*. The Homebush Bay site had significantly lower dissolved oxygen levels than either the Narrabeen or Lane Cove sites. The mean level of dissolved oxygen found at Homebush Bay (4.24 mg/l) is on the lower end of the scale for acceptable levels for resident populations of relatively tolerant freshwater fish species (3 mg/l; Alabaster and Lloyd, 1982). Some stations at Homebush Bay had extremely low dissolved oxygen levels (as low as 0.8 mg/l) which must be considered unacceptable. Dissolved oxygen levels can fluctuate dramatically in natural waters and few experiments have attempted to simulate natural conditions. It has therefore been suggested that further studies are necessary on the movement of fish during these processes (Alabaster and Lloyd, 1982).

5.4.1.6 Un-ionised ammonia.

No difference was found in un-ionised ammonia between sites. In addition no correlation was found between the presence/absence of *P. signifer* and the concentration of un-ionised ammonia at the 32 stations used in this study. Since un-ionised ammonia was of an appropriate concentration (Reddacliff, 1985; Cook, 1986 and Alabaster and Lloyd, 1982) this parameter is not implicated in the distribution of *P. signifer* in the current study.

5.4.2 Rainfall.

The salinity at Narrabeen was lower than that found at Lane Cove and Homebush which could be at least partially due to the higher rainfall at Narrabeen in December 1993, combined with the restricted access to the sea at this site. Prolonged low salinity at Narrabeen was observed after a period of heavy rain in the study reported in Chapter 3.

5.4.3 Abundance.

According to Calow and Petts (1992) there are three major factors that restrict our

ability to measure fish populations effectively: selecting methods of known accuracy, making allowances for temporal and spatial differences and taking environmental effects into account. This pilot study endeavoured to reduce temporal factors by sampling at all 4 sites within nine days of each other and 8 stations were sampled at each site to account for spatial differences within the sites. It should be taken into consideration that because of snags and substrate variability it was frequently difficult to catch *P. signifer,* particularly at some of the selected stations in Lane Cove. *P. signifer* could clearly be seen at the majority of stations at Lane Cove but could not be caught in the prescribed time. This was not the case at Homebush Bay, where when no *P. signifer* were caught, none were observed. There was a tendency to reduced total abundance of *P. signifer* at both sites in which *P. signifer* plus *G. holbrooki* were caught (Homebush Bay and Narrabeen) although it was not statistically significant. The mean numbers of male *P. signifer* at Mooney Mooney Creek (*P. signifer* not sharing habitat with *G. holbrooki*) was significantly larger than at Homebush Bay or Lane Cove. No relationship was found between the abundance of *P. signifer* and abundance of *G. holbrooki*, using either linear or nonlinear regression analysis.

By dividing the stations into those where *P. signifer* were present and those where they were absent it was determined that the pH, temperature and dissolved oxygen were all lower where *P. signifer* were present. Such conditions could be optimal for *P. signifer* whereas *G. holbrooki* have different optimal conditions. However no direct relationship was found between the presence of *P. signifer* and the presence of *G. holbrooki.*

No clear data were found to suggest that at the two sites where *P. signifer* were found alone (Lane Cove and Mooney Mooney Creek), individuals were any larger, more abundant or more reproductively active (in terms of gonad weight, fecundity or GSI) than at the sites where *P. signifer* were found with *G. holbrooki.* However, there were factors that differentiated one site from other sites. For example Narrabeen had lower salinity than two other sites and had the largest females of *P. signifer.* Male *P. signifer* from Homebush Bay had significantly larger GSI than those at all other sites. Possibly *P. signifer* males developing under conditions of poorer water quality have comparatively delayed testis development. On the other

hand the testis development may represent a compensatory increase in reproductive effort by *P. signifer* in response to *G. holbrooki* predation. Sillman *et al.* (1958) reported a size increase in fecundity for *Lebistes reticulatus* in response to removal of proportions of the population. There were significantly lower *P. signifer* at Homebush where both *P. signifer* and *G. holbrooki* were netted indicating that factors were present favouring the presence of *G. holbrooki* at this site. This difference in abundance could be primarily related to the more detrimental and varied water quality parameters and only secondarily to the presence of *G. holbrooki* at Homebush Bay.

Power Analysis

The difference in the mean abundance of male *P. signifer* at the Mooney Mooney Creek site and the Homebush site was 3.63. These sites were of interest as not only were we comparing the least disturbed site with the most disturbed site but *G. holbrooki* were caught at the most disturbed site (Homebush) but not at the least disturbed site, Mooney Mooney Creek. The statistical analysis of this study was found to have very low power. However, estimates of abundance and variation in abundance collected from this pilot study are useful for determining the design of a more elaborate study. By power analysis it was determined that it would be necessary to perform at least 25 samplings at each site to distinguish confidently differences between sites of the magnitude seen in this pilot survey. Such a large number of samplings could not physically be performed in the one day at each site and would therefore be open to temporal variation between sites.

5.4.4 Disturbed areas.

Homebush Bay was found to have the greatest variability in water quality parameters. It appears to be a very disturbed site. *G. holbrooki* have been found to be abundant where introduced species of grasses overhang the water whereas *P. signifer* prefer open water with weed bed habitats (Arthington *et al.*, 1983). Homebush Bay was the only location where there were no stations at which *P. signifer* only were caught. However, at the less disturbed Narrabeen location *P. signifer* alone were caught at some stations. The present study may lend support to

the earlier suggestion that *P. signifer* are more affected by habitat modification than by the presence of *G. holbrooki* (Arthington *et al.*, 1983).

5.4.5 The impact of exotic introductions.

A relationship between the presence of *P. signifer* and low levels of pH, temperature and dissolved oxygen was found when the 32 stations were analysed independently of sites. In addition abundance levels of *P. signifer* related positively with turbidity levels. The second of the two hypotheses of Douglas *et al.* (1994) to explain how exotic introduction affects native fish in the western United States of America, seemed to apply in the tank experiment of Chapter 4 **(p 179)**, viz:- displacement - where natives are displaced by exotics by predation, hybridisation (not relevant here) and competition. The first of the two hypotheses, viz:- replacement, where environmental deterioration actually removes the native fish leaving the area open for hardy exotic species, appears to be indicated by the results of this pilot study, although a more extensive field study would be necessary to confirm this. The presence of *P. signifer* appears to be related to water quality parameters and *G. holbrooki* are present and found in areas of poor water quality. If and when *P. signifer* are eliminated from an area by deteriorating water conditions *G. holbrooki* will be in a position to replace them.

Habitat modification provides conditions conducive to the growth and reproduction of *G. holbrooki* (Arthington *et al.*, 1983). Deep Creek at Narrabeen, as sampled in this 1993 study, had very few beds of *Zostera capricorni*. In 1985 *Z. capricorni* formed the habitat in which *P. signifer* were most commonly caught (Howe and Howe, 1991). *Zostera* thrives in intertidal zones where the substrate is composed of muddy-sand with substantial organic material (Hart, 1974). Changes in the abundance of *Zostera* beds may have been caused by pilot dredging operations at Billarong Reserve in 1986 (Erica Griffiths, pers. comm. Environmental Officer, Warringah Shire Council).

The change in habitat may form the first step in allowing the establishment of *G. holbrooki* before it replaces *P. signifer*. *Z. capricorni* beds would have been useful to *P. signifer* for three major purposes: as shelter; as a spawning substrate; and for the provision of food either directly or indirectly. In addition leachate (particularly

un-ionised ammonia) from Kimbriki tip, a waste disposal site has been found to be present in sufficient concentration to have detrimental effects on aquatic life downstream along Deep Creek particularly at high flow rates (Petrozzi, 1993). *P. signifer* prefers a weedy environment in flowing water close to shore where it feeds on aquatic insects (Ivantsoff, 1980). Like *Carassius auratus* (goldfish), *P. signifer* ovulate in response to a specific temperature and photoperiod, but in addition goldfish ovulate in response to aquatic vegetation (Stacey *et al.*, 1979). It is very likely that aquatic plant material (*Z. capricorni*) also induce *P. signifer* to breed and that the reduction of this vegetation could be detrimental to *P. signifer*. *P. signifer* will show increased spawning in response to introduction of plant material under aquarium conditions (pers. obs.).

5.4.6 Impact of *Gambusia* on various species of fish.

From a management perspective it is necessary to know the degree to which a change in one species affects other species (Calow and Petts, 1992) and following from this it is of importance to fully understand the impact of introduced species. Many studies in other countries have shown that *Gambusia* are detrimental to native populations of fish (Meffe, 1985; Galat and Robertson, 1992). *Poeciliopsis occientalis* (Sonoran Topminnow) has become endangered in the southern part of Colorado and this has been attributed to the introduction of *Gambusia affinis* (Schoenherr, 1981). In a field study in Southern Nevada more *Crenichhthys baileyi* (native to the area) were collected per unit effort, in the absence of *G. affinis* (Deacon *et al.*, 1964). Predation by *G. affinis* on *P. occientalis* has been suggested as the method of replacement (Meffe *et al.*, 1983). *G. holbrooki* were found to have a detrimental impact on *Heterandria formosa* (least killifish) by predation (Schaefer *et al.* 1994). On the other hand, Pen and Potter (1991) in their field study at the Collie River in southwestern Australia, found no evidence that *G. holbrooki* ate eggs, juveniles or adults of three other native species found in the river system. They appear to be able to cohabitate with the other indigenous species because they have different spawning times (the natives breed earlier and are large enough to avoid predation when *G. holbrooki* are abundant) at different localities (Pen and Potter, 1991). *P. signifer* and *G. holbrooki* however, have similar spawning times

that can be in the same locality, indicating strong potential for destruction of *P. signifer* and related egg laying species.

5.4.7 Potential for restricted Pseudomugilidae.

The present study was carried out in open expanses of water and not in areas where *P. signifer* might be found in restricted habitats (for example, tidal flats in the Ross River, North Queensland). *Scaturiginichthys vermeilipinnis* (the redfinned blue-eye) have only been found in five small shallow pools (described as puddles) in the Lake Eyre Drainage System (Ivantsoff *et al.* 1991). *G. holbrooki* have been found in four out of the five ponds (Unmack and Brumley, 1991). If the study were to be repeated in such a situation, with more extensive sampling, as indicated from the power analysis of the results of this pilot study, it is possible that results would be more comparable to that of the tank study in the previous chapter. Poor water quality conditions, particularly at the Homebush Bay site, are of major importance as these appear to be the stepping stone that is required by *G. holbrooki* before they replace native species.

Chapter 6.

General Discussion.

6.1. Background.

Many facets of the biology and ecology of Australian native fishes are poorly known. The selection of an appropriate strategy for the conservation and management of threatened and non-threatened species is constrained by the lack of basic biological information. As Wager and Jackson (1993) commented "Approximately 195 freshwater fish species and subspecies have been formally described from Australian waters. A further 22 undescribed taxa are currently recognised. Approximately 8% of Australian freshwater fishes are threatened with extinction, and about 25% are considered to have either significantly declined in distribution, or are found in restricted areas."

The current project was carried out to increase our knowledge of the genus *Pseudomugil* generally and specifically in certain aspects of the biology and reproductive characteristics of *P. signifer*. In addition, an investigation was undertaken to determine the impact of the exotic species *G. holbrooki* on the growth and reproductive capacity of *P. signifer*, as *G. holbrooki* has been considered a possible threat to *P. signifer*. The information gained on the biology and reproductive characteristics of *P. signifer* is not only of interest from a biological perspective but should be of value for management and conservation purposes.

6.2 Major findings.

In this project an endeavour has been made to identify factors affecting the reproductive biology and growth of *Pseudomugil signifer*, and in this way to gain a more complete picture of the life history of the species than has previously been available. The data collected showed:-

(i) Relationships among species of the genus *Pseudomugil*, by observing the breeding behaviour and normal egg surface (SEM) and embryological morphology of *P. signifer* and making comparisons with three other Australian species of *Pseudomugil*.

It was found from these comparisons that relevant information obtained for *P.*

signifer could be extrapolated for management and conservation decisions, to other species from the genus. While the species could be distinguished by various features of their embryology, the species of *Pseudomugil* studied were sufficiently similar to allow extrapolation of studies in *P. signifer* to the other pseudomugilids. The embryology of *Pseudomugil* species distinguishes them from Melanotaeniidae.

It is necessary to have knowledge of the fundamental breeding biology and taxonomy of a species before any interpretation of factors that might affect their reproduction can be assessed. Part of the main aim of this thesis was to examine the impact of *G. holbrooki* on *P. signifer*. Unlike that of *P. signifer*, the life history of *G. holbrooki* has been studied extensively.

(ii) That *P. signifer* had a seasonal reproductive cycle and that there were relationships between its cycle and physical and chemical environmental characteristics.

Reproductive development occurred in a similar manner in captivity and in the wild (breeding commenced as the temperature and daylength increased and declined as the daylength and temperature decreased). The findings confirm the reproductive biology of this species and provide more scope for experiments to be conducted in captivity.

(iii) That *P. signifer* was threatened by *G. holbrooki* under the controlled conditions of a tank experiment (reduced growth and fecundity). A pilot study in the field determined the amount of sampling required for a more extensive field study on the impact of *G. holbrooki* and physical and chemical factors on *P. signifer*.

Differences in abundance of *P. signifer* in the field appeared to be related to water quality parameters. Human intervention has caused deteriorating water quality in river systems (runoff, channel clearance and riparian clearance). Human activity has also resulted in the widespread introduction of *G. holbrooki*. It has been suggested that *P. signifer* is affected more by habitat alteration than by the presence of large populations of *G. holbrooki* (Arthington *et al.*, 1983). *P. signifer* prefer a weedy environment in flowing brackish water close to the shore (Ivantsoff, 1980) whereas *G. holbrooki* are most frequently found in warm water that is not fast flowing (Merrick and Schmida, 1984). The clearance of seagrass beds which have occurred at Deep Creek, Narrabeen makes the area less conducive to the successful

existence of *P. signifer*.

(iv) Comparison of fecundity of *P. signifer* between the tank study and the field study.

In the tank experiment the fecundity of *P. signifer* was found to be greatly reduced in the presence of *G. holbrooki*. However, in the pilot field study no significant difference was found in the fecundity of *P. signifer* between the sites where *G. holbrooki* were present or absent. *G. holbrooki* have been positively associated with disturbed sites (Arthington *et al.*, 1983). Examination of the relationship between ovarian weight and body weight, and fecundity and body weight showed a relationship between these parameters in the field which was only mirrored in the control groups which did not receive supplementary feeding.

(v) Some possible factors leading to reduced activity of ovaries of *P. signifer* in the tank study.

The results of these studies suggest that one or more of the following factors may have ultimately led to the reduced activity and size of the ovary in *P. signifer*:-

Interruption of normal behaviour patterns

Endocrine response

Physiological stress

Stocking density

Territoriality.

It can be postulated that there was possible interspecific competition for space in the tanks resulting in stress inflicted by *G. holbrooki* on *P. signifer*. Use of the modified schematic diagram of McLusky, 1984 shows the sequence of events in Fig. 6.1. Stress would be detected first by the nervous system. At this stage, in the wild, *P. signifer* could hypothetically seek cover (which was difficult in the confined space) and thus recover. Continued stress would eventually lead to endocrine effects. The response to stress in vertebrates is mediated by the hypothalamus-pituitary-adrenocortical axis (Fletcher, 1981), resulting in increased secretion of corticosteroids and catecholamines (Mazeaud *et al.*, 1977). Under these circumstances, cardiac volume increases with the secretion of catecholamines causing an increase in aortic blood pressure (Mazeaud and Mazeaud, 1981).

In freshwater fish a diuretic response has been found to result from the increased

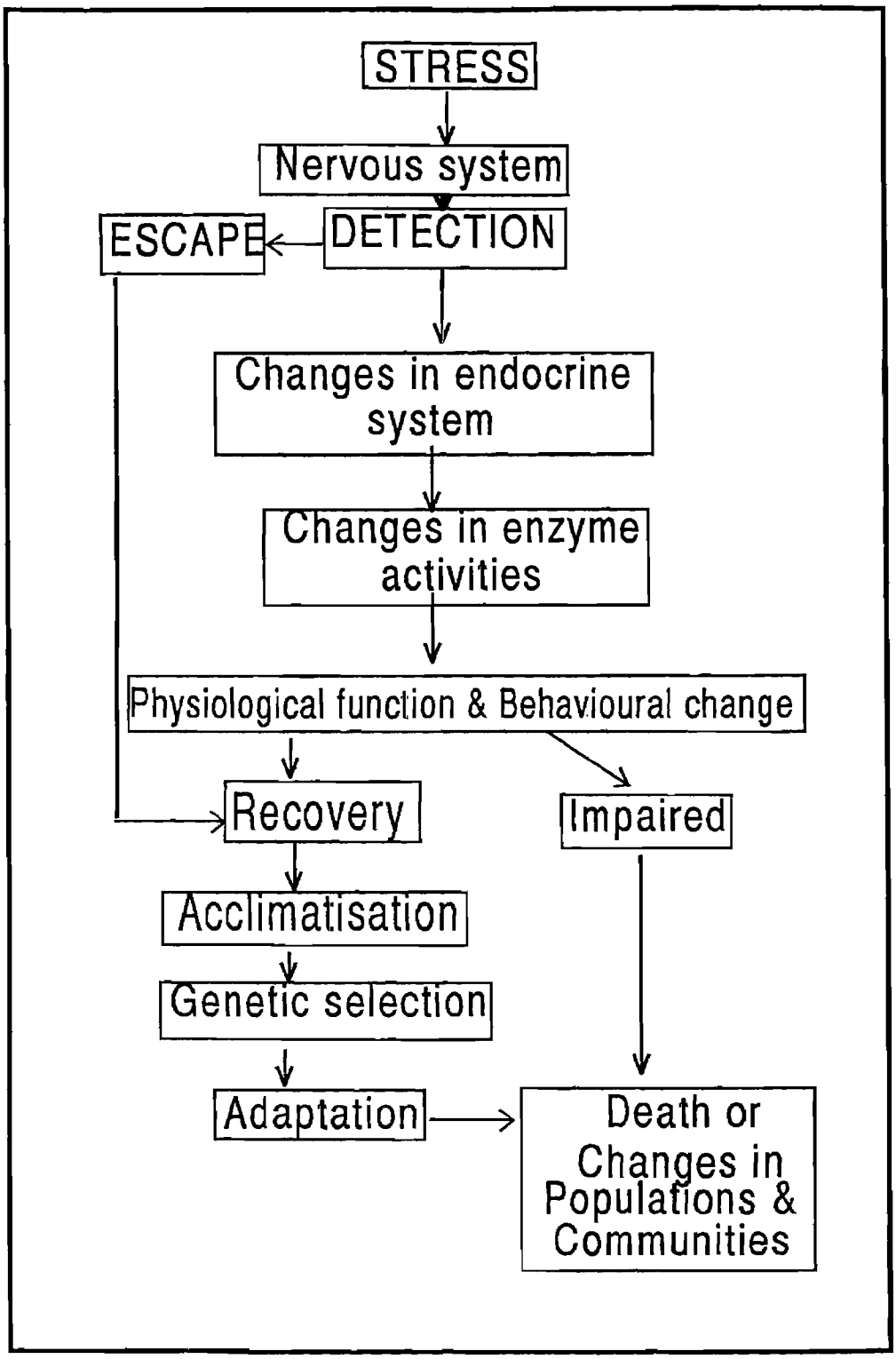


Figure 6.1 Schematic sequence of the effects of environmental stress on estuarine animals (modified from McLusky, 1989 after Blackstock, 1984).

cardiac output (Rankin and Babiker, 1981). Glucosteroids released under stress affect the biosynthesis of reactant proteins (Fletcher, 1981). Changes in enzyme activities in turn cause physiological and behavioural changes.

The response to stressors may enhance the ability of *P. signifer* to escape and recover in the wild, but the majority of *P. signifer* housed with *G. holbrooki* did not recover. They either became impaired (lack of growth and ovarian development) or died. The impact in the tank experiment was largely on the adult fish, exhibiting caudal fin nipping, failure to grow and failure of the ovary to produce viable eggs. The major difference between the field and tank study may simply be that in the field, fish could generally escape whereas in the tanks *P. signifer* were confined with *G. holbrooki* and were not able to escape. Changes to communities result, according to the ability of individuals to adapt. In a confined space the fish were probably unable to resist stress and it was beyond the capacity of the individual organisms to adapt to such an abrupt change. The current field study was performed in open expanses of water but *P. signifer* do occur in restricted habitats such as tidal flat ponds in the Ross River, North Queensland (pers obs.) and *S. vermeilipinnis* have been found in very small ponds with *G. holbrooki* (Unmack and Brumley, 1991). Where pseudomugilid species are found in such confined circumstances in the wild, it could be expected that similar stress factors might be important as were found in the tank experiments, but no results of this nature have been published. Ultimately stress effects on individuals of the genus *Pseudomugil* by *G. holbrooki* would then be expected to lead to changes in populations and communities, either by particular fish escaping, leading directly to differences in the population or by the death of individuals. In this manner, the selection pressure on *P. signifer* caused by the presence of *G. holbrooki* could alter the population structure.

(vi) No doubt as a consequence of the ovary of *P. signifer* failing to develop, recruitment of young *P. signifer* did not occur when *G. holbrooki* were housed with them in the tank experiment. Recruitment of young however, was observed when *P. signifer* were held alone. No data were collected on this aspect in the field, although juvenile *P. signifer* were observed at all sites.

6.3 Strategies used by *P. signifer* and *G. holbrooki.*

It is important for both conservation and management reasons to determine the long term future of *P. signifer* as a representative of the family Pseudomugilidae and to determine whether this species will have the resilience to survive and reproduce in generations to come. Fish must have the physiological mechanism to be able to detect environmental cues and respond to the change; the integral elements of a reproductive strategy include age at which reproduction starts, post breeding survivorship, size-specific fecundity, the organisation of reproductive behaviour and the timing of the breeding season (Potts and Wootton, 1984).

Since this project has shown that *G. holbrooki* can have a marked deleterious influence on *P. signifer* only under tank conditions, a comparison is made of the strategies used by the two fish species which will provide some insight into why *G. holbrooki* appears to be more successful than *P. signifer.*

6.3.1 Reproduction.

Prespawning, spawning and postspawning behaviour - The prespawning, spawning and postspawning behaviour of *P. signifer* has been summarised in Chapter 2. Males of *G. holbrooki* in spawning condition increase the brightness of their colouration and extend their ventral fins in various directions, frequently forward of their mouth. The female with no brightening of coloration, meanwhile darts in and out of surface foliage with the male in pursuit (Gale, 1915). When the male finally reaches the female he inserts his gonopodium into the female's oviduct. Both species were found to breed over the summer season in the Sydney region. *P. signifer* were found to produce between 2-7 eggs per day (Chapter 2). They tended to spawn for several days and then have a few days break. The fecundity results for *P. signifer* presented in this thesis show an average fecundity in December in field specimens of 9 whereas average fecundity for *G. holbrooki* in field specimens was 45. A mature female *G. holbrooki* can produce 5 broods in a season; the young from these broods can themselves become reproductively active from the age of 4-6 weeks (Krumholtz 1948). *G. holbrooki* thus has a higher reproductive potential than *P. signifer* (that is *G. holbrooki* has a much larger chance of producing more offspring than *P. signifer*). In one breeding season a single female *G. holbrooki* could produce

upwards of 200 young (based on 5 broods) with an initial sex ratio of 1:1 (Krumholtz, 1948). The first generation of 100 females could give rise to two broods in the same season producing a conservative total of another 9,000 offspring (two broods x 100 fish x 45 eggs). A single *P. signifer* could probably only produce a maximum of 700 eggs in a 5 month breeding period (based on a sixteen day period to hatch, over 5 months at 7 eggs/day with 30 days of rest). In addition the young of *G. holbrooki* are produced live and are a much larger size (8-9mm) than newly hatched *P. signifer* (5mm) (making *P. signifer* more easy prey). The eggs of *P. signifer* are externally fertilised and therefore the male fish has to be present and fertilise each of the eggs as they are liberated into the water. Eggs can remain unfertilised because the spermatozoa does not successfully enter the micropyle of the egg (pers. obs.).

Koehn and O'Connor (1990) suggest that there are two strategies used in fish reproduction: "Many eggs no care" and "Few eggs plus care". *G. holbrooki* could perhaps be placed in the latter group but *Pseudomugil* species should be placed in an additional third category, that of "Few eggs no care".

In *G. holbrooki* the sperm from a single mating is preserved in the oviducts, and several successive broods are fertilised and produced without the presence of any male (Constantz, 1989). The fact that the male *G. holbrooki* does not have to be present at the birth of the young and in fact fertilises several broods of eggs with one insemination presents a major reproductive advantage over *P. signifer*. *G. holbrooki* do not have to spend as much time and effort with courting displays and rituals. Because *G. holbrooki* are lecithotrophic livebearers a single female can deposit a potential breeding population at the edge of the existing range (Rosen and Bailey, 1963). *P. signifer*, however, must have at least a breeding pair to establish new populations at or beyond the fringe of the species range. *G. holbrooki* therefore have a selective advantage over *P. signifer* in extending their breeding range.

The peak of the reproductive effort in *G. holbrooki* has been found to be early in the season when food is plentiful (Lloyd *et al.*, 1986). Data are not available on this aspect of the biology of *P. signifer*.

Knowledge of the effects of temperature on spermatogenesis in *P. signifer* is deficient. However, it has been recently found that mild temperatures alone are not

sufficient for meiosis and spermiogenesis in *G. holbrooki* (a long photoperiod is also required) (Fraile *et al.*, 1994).

Gambusia affinis and *G. holbrooki* are very closely related and are commonly named the mosquitofish (this name being contentious since they are not the only fish that eat mosquito larvae). The species found in Australia is *G. holbrooki* (Wooten *et al.*, 1988) but frequently has been referred to as *G. affinis*. It has been found that *G. affinis* store lipid in their somatic cells and stored energy as a result of this, can be transferred to the ovary to aid in reproduction (Reznick and Braun, 1987). The lipid storage cycle found in *G. holbrooki* is thought to be a reproductive adaptation to winter conditions (Meffe and Snelson, 1993). No studies on lipid storage have been conducted on *P. signifer*. If *P. signifer* do not have such a mechanism or the mechanism is not as efficient, *G. holbrooki* may be reproductively advantaged.

These findings suggest that reproductive behaviour does not play as significant a role in the reproductive success of these two species as the physiological adaptations exhibited by the two species.

6.3.2 Comparative size of the two species and growth rates.

P. signifer males have been reported up to a length of 70mm (Merrick and Schmida, 1984); however, the common range is 25-45mm (Ivantsoff, 1980). *P. signifer* used in the tank study and caught during the field study reached a maximum of 35mm. *G. holbrooki* males are very much smaller (maximum length of 35mm) than females (60mm). Because females of *G. holbrooki* are the more aggressive of the two sexes, as observed by predation in the laboratory behaviour experiment (Chapter 4, Schoenherr, 1981 in *G. affinis*), and they are larger than *P. signifer*, they have an obvious advantage over *P. signifer*.

G. holbrooki grow at a faster rate than *P. signifer* and can reach sexual maturity as early as 4 weeks of age (Krumholtz, 1948) whereas secondary sex characteristics were not observed in *P. signifer* until six months to a year of age (Chapter 2). Since *P. signifer* are seasonal breeders (Chapter 3), they cannot breed until the season after they were spawned.

6.3.3 Territoriality.

It has been found that male *P. signifer* are territorial during the breeding season (Semple, 1986 pers. obs.). The literature is almost silent on whether *G. holbrooki* is territorial. However, in experiments with *Aphanius mento* (a territorial species) and *G. affinis* in aquaria, it was found that female *G. affinis* staged counter attacks on *A. mento* resulting in the death of *A. mento* (Sharma *et al.*,1985). This attack could be viewed more as an act of aggression than one of territoriality on the part of *G. affinis*. Lloyd (pers. comm) does not believe there is any evidence that *G. holbrooki* is territorial. If this is so, *P. signifer* may be at yet another disadvantage compared with *G. holbrooki* in that the males allocate more energy and time defending their territory against both conspecific and foreign aggressors and thus reducing their allocations to feeding and reproductive activities. In addition they become more stressed. It was found that *Tilapia zillii* juveniles passively interfere with male *Cyprinodon* because the latter constantly drive out juvenile *Tilapia*, therefore spending considerable time guarding their territories while less time is spent courting and so reproductive success is lowered (Schoenherr, 1981). Although *G. holbrooki* are aggressive towards *P. signifer*, they do not defend a specific territory. This strategy allows them to allocate sufficient time and energy for feeding and breeding activities. It has been suggested (Schoenherr, 1981), that aggressive behaviour is an expression of territoriality. If this is the case *G. holbrooki* could be regarded as territorial, but apparently over an indefinite range.

6.3.4 Aggression.

Individuals of *P. signifer* have been reported to be very aggressive (Leggett and Merrick, 1987). Males of *P. signifer* from northern Queensland were found to be extremely aggressive when more than one male was housed in an aquarium (Semple, 1986). Among the behavioural interactions that cause niche partitioning in nature is aggression (Schoenherr, 1981). The loser is expelled from a territory and resource utilisation is maximised by the conqueror. *G. holbrooki* is known to be very aggressive, attacking and nipping the fins of other fish (Minckley and Deacon, 1968; Grant, 1978; McDowall, 1980; Lloyd, 1984; Lloyd, 1990 and Wager and Jackson, 1993). In the current behavioural aquarium experiments a behavioural hierarchy was

observed. Female *G. holbrooki* dominated overall (observed chasing adult *P. signifer* and preying on young *P. signifer*). Male *G. holbrooki* followed the female in aggressive tendencies. Male *P. signifer* could be considered next in line in aggressive tendencies, but this aggressive behaviour appeared to be directed towards guarding its territory. Last on the pecking order were female *P. signifer* which suffered the greatest mortality among adult fish. In these behavioural experiments the number of female *P. signifer* had declined from six to zero by harvest, but two of the three males had survived. On the other hand all of the three male *G. holbrooki* and five out of the six female *G. holbrooki* survived until harvest.

6.3.5 Predation and cannibalism.

Predation has been suggested as the main mechanism by which *G. holbrooki* deplete native species of fish (Courtenay and Meffe, 1989). Predation of juvenile *Poeciliopsis occientalis* by *G. affinis* was found to be the main mechanism by which it replaced *P. occientalis* in field and laboratory experiments (Meffe, 1985). They have the anatomy that enables them to be successful predators (Meffe *et al.*, 1983 and Rosen and Mendelson, 1960). *Gambusia* have open cephalic canals and strong conical teeth (Rosen and Mendelson, 1960) which implies a preferred carnivorous habit. Open canals are thought to aid in detection of moving prey in surface waters (Meffe and Snelson, 1989). *Pseudomugil* species have teeth which are conical to caniniform (Allen and Cross, 1982) but no information was found in the literature as to whether they have open cephalic canals. *G. holbrooki* have been observed to attack larger fish species by caudal fin nipping, and to eat smaller fish (Meffe *et al.*, 1983; this study Chapter 4) and juveniles (Meffe, 1985; this study Chapter 4). Meffe (1985) predicted that a female *G. holbrooki* could easily eat all young produced by one *P. occidentalis* and concluded that even rare predation on fry could have detrimental consequences on species with low fecundities. In the current study it has been confirmed that *P. signifer* has a much lower fecundity than *G. holbrooki*.

In two separate studies on the interaction between *Heterandria formosa* and *G. holbrooki* (similar sized closely related sympatric species found in the southeastern coastal plain of the United States), it was found that size selective predation not competition (large *G. holbrooki* preyed on small *H. formosa*) was the dominant

interaction (Belk and Lydeard, 1994 and Schaefer *et al.*, 1994). One study was conducted in artificial pools (Belk and Lydeard, 1994) and the other was a field study (Schaefer *et al.*, 1994). Intraguild predation (IGP) is defined as "the killing and eating of species that use similar resources and are thus potential competitors" (Polis *et al.*, 1992). The study by Schaefer *et al.*, (1994) suggested that asymmetrical intraguild predation occurred. Large *G. holbrooki* are capable of preying on juvenile *H. formosa* whereas large *H. formosa* apparently do not prey on small *G. holbrooki*. It would be of interest to determine whether similar interactions are found with *G. holbrooki* and *P. signifer*.

G. affinis have also been found to greatly reduce the number of invertebrates (rotifers, crustaceans and insects) in artificial pools, leading to an extremely large increase in phytoplankton populations, causing ecosystem alteration (Hurlbert *et al.*, 1972; Hurlbert and Mulla, 1981). Both studies performed in the laboratory or ponds have suggested that *Gambusia* are cannibals (Walters and Legner, 1980; Harrington and Harrington, 1982). Nesbit (1993) found cannibalism by *G. holbrooki* to be very low in nature (1.15%) and warned against the use of laboratory results for drawing conclusions of an evolutionary nature and selective benefits.

6.3.6 Fitness of the species in relation to their evolutionary development.

As pointed out in the introduction of this thesis *G. holbrooki* have a lower proportion of males than females in the wild (Krumholtz, 1948). This probably has little impact on reproduction at the population level as a single male can fertilise many females and females can store sperm in the ovary and therefore a single insemination fertilises several broods (Constantz, 1989). Field data collected over the period of this project provide evidence that there are also fewer male *P. signifer* than females in the wild (one male to two females) but *P. signifer* does not have the same reproductive mechanisms and males are required for fertilisation at every spawning. This provides an evolutionary advantage to *G. holbrooki* over *P. signifer*.

It has been found that female *G. holbrooki* in the field only rarely carry broods with unfertilised ova (Snelson, 1989). It is plausible that external fertilisation of eggs as in *P. signifer* would produce a larger proportion of infertile eggs.

G. holbrooki are large at birth compared with *P. signifer* on hatching. In addition,

newly hatched *G. holbrooki* immediately begin to feed. Newly hatched *P. signifer* do not begin to feed immediately (pers. obs.), and are presumably relying on reserves of yolk.

The estuarine environment is considered to be one of the most productive regions in the world (Schelske and Odum, 1961) and estuarine fish have had to adapt to rapid and large changes in conditions inherent to these surroundings (Dando, 1984). *G. holbrooki* appears to have a better ability to adapt to changed environmental conditions than *P. signifer*. Populations of *G. holbrooki* that had been exposed to extremely high temperatures for 28 years responded physiologically by maturing quickly and reproducing at a small size. *G. holbrooki* thus has been considered to be a highly plastic species (Meffe, 1992). They are able to develop along different paths in varied environments (Trexler, 1989). This type of adaptive experiment has not been carried out on *P. signifer* or related species.

It is not just that *G. holbrooki* are livebearers which make them successful, as many other groups of fish are livebearers but not as successful (Wourms, 1981; Meffe and Snelson, 1989b). Additional ecological studies are required to define specifically the adaptations that have made *G. holbrooki* (and all Poeciliidae) successful.

6.3.7 Genetic diversity.

It has been suggested that genetic diversity should be the focal point of conservation and management issues (Smith *et al.*, 1989). Intraspecific genetic variability can determine whether a species can successfully adapt to environmental conditions in the future (Smith *et al.*, 1989). Robbins *et al.* (1987) found that genetic variability among the *Gambusia* is among the highest among vertebrates. *Gambusia*, therefore, can probably successfully adapt to changes in environmental conditions. It is difficult to ascertain from the literature whether *P. signifer* has the same genetic variability of *G. holbrooki* as no studies along these lines have been performed. The only study on the genetic variability of *P. signifer* suggests that the Cairns, Queensland tropical population and other populations down the east coast of Australia show a clinal variation but the amplitude of individual variation is unknown (Hadfield *et al.* 1979).

6.3.8 Geographic isolation of Australia.

The limited, and frequently unreliable freshwater systems on the present-day Australian continent contain a depauperate freshwater fish fauna (McKay, 1984). Since the breakup of Pangaea 160 million years ago (Ma), the continent of Australia became progressively more isolated (Powell, *et al.*, 1981; Veevers, 1991). The Eastern Gondwana fragment separated, containing Australia, Antarctica and India, with some connection to South America maintained through West Antarctica, although the present day world distribution of fish orders suggests an effective barrier between East and West Gondwana (McKay, 1984). India was effectively fully separated from Australia by the late Cretaceous, 96 Ma. Volcanic activity and changes to the tectonic activity along the north-eastern border led to the emergence of the western part of Australia from a shallow sea and the establishment of the asymmetric drainage pattern that has characterised Australia since (Veevers, 1991). Spreading between Australia and Antarctica finally created a substantial barrier by the late Miocene, about 10 Ma. Northward movement brought northern north-western Australia into collision with Pacific terranes in the east and the complex Indonesian arc in the west during the period to 5 Ma, in the process starting to bring the Australian region back into contact with other land masses (Veevers, 1991; Burrett, *et al.* 1991).

Climate evolved along with the plate tectonics (Kemp, 1981). Overall, a cool to warm, moist temperate climate predominated through much of the Tertiary, with widespread rainforests (Kemp, 1981, McKay, 1984). With the opening of the seaway between Australia and Antarctica during the Eocene, a cooling trend appeared, and in the late Miocene a major cooling event led to drying of the Australian climate, with grasslands spreading. Repeated and geologically abrupt climatological changes during the Pleistocene, with major fluctuations in sea level, led to many faunal extinctions through alternate inundation and periods of glaciation with extreme aridity (McKay, 1984; Galloway & Kemp, 1981).

Although not diverse, the fish fauna represents diverse origins (McDowall, 1981; McKay,1984).

The elements of the fauna are seen by (McDowall, 1981) as;

1. Old endemics - *Neoceratodus* and *Lepidogalaxias* from their monotypic families

and the osteoglossid *Scleropages*.

2. Pantropical species - Synbranchid eels, Australian freshwater ariid catfishes.

3. Groups with southern-temperate relationships - found in cool waters of at least a couple of Australia, New Zealand, South America and South Africa, these include some lampreys, retropinnids, prototroctids, galaxids and aplochitonids. All these have at least some members with marine stages.

4. Obvious widespread Indo-pacific relationships. A large group of families, all with marine members. Atherinids fall into this group. Melanotaenids are endemic to Australia/New Guinea and viewed by McDowall (1981) as atherinomorph in origin.

5. Uncertain origins: including members of the Perchthyidae, *Maccullochella, Percalates*. McKay (1984) feels that these would have been established at least by the time Australia started to separate from Antarctica.

When Australia started to drift north from Antarctica 50 Ma it took vestiges of the Paleozoic and Mesozoic heritage, including older subtropical elements with relationships with Africa and India, and a series of southern cold-adapted forms that characterised the cool temperate Antarctic and southern South America in the Eocene. Because of the different climatic adaptations these groups have remained distinct, yielding a specific group of southern fish, a middle group dominated by the percichthyids and a more diverse northern fauna of various origins (Keast, 1981; McDowall 1981).

Because of geologic and climatic events Australian freshwater fish species have developed in isolation and are not as specialised as those in regions with a greater diversity of species (McKay, 1984; Douglas *et al.*, 1994). Such a "permissive" fauna is at greater risk of damage by the introduction of fish such as *G. holbrooki* which have evolved in association with a more diverse fish fauna (McKay, 1984).

Courtenay and Meffe (1989) suggest that *G. holbrooki* fulfil seven out of the eight criteria suggested by Ehrlich (1986) as successful invaders which include:-

abundant in original range

polyphagous

short generation time

a single female can colonise new habitats

broad physiological tolerances

closely associated with human beings, and

high genetic variability.

The one character that *G. holbrooki* does not comply with is being large in size. Two additional characteristics that make them successful are specialised reproduction (livebearers, several broods/ year) and high aggression levels (Courtenay and Meffe, 1989).

6.3.9 Summary of possible survival advantages *G. holbrooki* has over *P. signifer.*

- *G. holbrooki* has the ability to produce over ten times the offspring of *P. signifer* in one year, (based on figures from present studies).
- *G. holbrooki* females store sperm which can be used to fertilise several broods, whereas *P. signifer* females produce eggs that require fertilisation by the male at each spawning.
- Average fecundity of female *G. holbrooki* (30) is much larger than that of *P. signifer* (9).
- *G. holbrooki* produces live young whereas *P. signifer* produces eggs.
- *G. holbrooki* young are larger at birth than *P. signifer* are at hatching.
- *G. holbrooki* has larger, more aggressive females.
- *G. holbrooki* does not appear to be territorial whereas male *P. signifer* is.
- A one male to two female sex ratio of both species in the wild would perhaps give *G. holbrooki* a selective advantage. Once the male has fertilised the female he is not required for the rest of the year. However, the male *P. signifer* has to be present at each spawning.
- As Australian freshwater fish species have evolved in isolation *P. signifer* may not be as proficient in competitive skills or predator defences as *G. holbrooki*. This has been suggested to be the case for indigenous fishes of Western North America (Douglas *et al.*,1994) and a similar situation is thought to have occurred in Australia (Crowl *et al.*, 1992).
- *G. holbrooki* appears to have a better ability to adapt to changed environmental conditions than *P. signifer. G. holbrooki* appears to have a wider tolerance to many water quality parameters than *P. signifer* and the former are frequently

found in disturbed habitats.

In summary, *P. signifer* is very different from *G. holbrooki*, both from an evolutionary and a physiological perspective.

6.4 Need for further research.

Further research in this field should encompass extensive, empirical data collection with experiments and modelling. In particular, the following areas should be covered.

- Further field work using fish trapping and other forms of netting (which would provide more accurate data) would be useful in clarifying information on the life history of *P. signifer*, such as their life span, since this information is required for management purposes.
- Information on whether or not *P. signifer* may be considered a 'plastic' species (as *G. holbrooki* is) may be of value. A genotype which produces a variable phenotype under differing environmental conditions is said to show phenotypic plasticity (Hartl and Clark, 1989). It has been suggested that information on the plasticity of a species could be used as a conservation tool to specify limits to survival and reproduction before recovery or protection programs are commenced (Meffe, 1992).
- More controlled captive experiments would be useful to determine whether one or both of the environmental factors, daylength and temperature, are the cues for breeding in *P. signifer*. From a management aspect it would be of particular interest to determine whether temperature alone is the cue as human activities (dams, diversions and discharges) can lead to changes in water temperature.
- Some studies have determined the relative abundance of *P. signifer* (Arthington *et al.*, 1983; Chapter 5 present study) but there do not appear to be any reasonable estimates of population size or density. Population size has not been measured in *G. holbrooki* (Meffe and Snelson, 1989b). There are several reasons for this:- No mass marking techniques have been developed for pseudomugilids or *Gambusia*. *P. signifer* and *G. holbrooki* populations are generally large and temporally variable making it necessary to tag a large

number of fish to obtain reasonable estimates which would be difficult from a practical point of view. Both species also inhabit variable habitats including tidal pools, mangrove areas with many snags and variable substrates and water of various depths. Field studies at sites with both *P. signifer* and *G. holbrooki* and sites with *P. signifer* only could clarify whether the presence of *G. holbrooki* reduced the abundance of *P. signifer*. The sampling method used in the current study was made more difficult with different substrates and snags.

- Further field work might show more chronic impacts of *G. holbrooki* on *P. signifer*. The tank experiments showed acute effects (with non-growth and reduced fecundity of *P. signifer* when it was housed with *G. holbrooki*). Captive and field experiments could provide further information into the stress relationships between *P. signifer* and *G. holbrooki*. Co-existence experiments with the other three species of *Pseudomugil* and *G. holbrooki* could also be performed to compare differences in response within the genus.
- There is a need to promote parallel studies in the field and laboratory. Field studies have shown that *Gambusia* is frequently successful in environments that are detrimental to other fish but it is not known what adaptations allow this species to succeed in such habitats (Meffe and Snelson, 1989). It would be very useful to conduct laboratory experiments on the physiology and behaviour of *P. signifer* and *G. holbrooki* across a variety of habitat conditions and in the presence of various stressors.
- Many more data are available on the mechanism of reproductive action and genetics of *G. holbrooki* than *P. signifer*, or for that matter for any other small fresh water or estuarine Australian fish species. It would be a great advantage to learn more about the endocrinology and genetics of our native species. Additional knowledge, particularly on genetic variability would help in the management of the species. In the face of the increasing number of anthropogenic impacts on its habitats, data should be collected on annual and seasonal changes in effective population size and in the genetic and demographic composition of populations.

It would also be pertinent from a management aspect to determine

more about the chemical that *G. holbrooki* release to stimulate male sexual behaviour as found by Amouriq (1964). It is possible that greater knowledge about it could aid in the management of *G. holbrooki* by, for example, interfering with the taste perception of the male as it is thought the male perceives the chemical via taste (Parzefall, 1973).

In summary, we need to design field and laboratory experiments to increase our knowledge of the genetics and life history of *P. signifer* in terms of their population biology and evolution and evaluate the influence of environmental variables and possible chronic effects of *G. holbrooki* on these populations.

6.5 Management.

Ten of 24 Recovery Outlines proposed in an Action Plan for Australian freshwater fish name exotic fishes as the major threat (5 list *G. holbrooki* and 5 the brown trout *Salmo trutta*) (Wager and Jackson, 1993). 14.5% of New South Wales freshwater fish species are alien to Australia (Faragher and Harris, 1995)

G. holbrooki need to be managed. How? Educate the public not to release them into waterways or move them from one water way to another. It would be very difficult to eradicate *G. holbrooki* unless one sex could be made infertile by some means. However, any chemical put in the waterways to induce infertility may affect other fish. Plans could be put into action to curtail any extension of *G. holbrooki*'s distribution in Australia. It would be extremely difficult to eradicate all *G. holbrooki* without damaging native populations of fish. Unless all female *G. holbrooki* are eradicated from a water way this environmental strategy would not be successful for two reasons. Firstly *G. holbrooki* are lecithotrophic livebearers and produce several broods in a season without the presence of the male. Secondly poeciliids appear to increase their reproductive effort in response to heavy predation (Meffe and Snelson, 1989). In addition, in a study using *Heterandria formosa* (the least killifish) with different starting densities of *G. holbrooki* (in artificial tanks) it was found that the presence of *G. holbrooki* at any density had a negative effect on the population of *H. formosa* (Lydeard and Belk, 1993). These data strongly support the complete removal of introduced *G. holbrooki*.

Galat and Robertson (1992) suggested 3 strategies for the control of *G. affinis* 1) physical modification of habitat to prevent reinvasion; 2) eradication of existing *G. affinis* populations and 3) educating the public of the value of native fish and their effectiveness as mosquito control agents.

In addition the public should be made aware of the consequences of moving *G. holbrooki* from one waterway to another (one female could populate a waterway that previously carried no *G. holbrooki*). Information should be provided to the public with illustrations and facts about *G. holbrooki* to persuade them not to release *G. holbrooki* into the wild.

One possible method to eradicate *G. holbrooki* from an area might be to choose one that is known to have a monoculture of *G. holbrooki* because of disturbance and poor water quality and eradicate the fish by rotenone. After this a concerted effort would be required to rehabilitate the water body and then introduce the native fish and plants back to the water system. The dynamics of the life cycle of *P. signifer* is such that a maximum of 700 young could only be produced in one year by a single female. The newly hatched larvae are very vulnerable as they are small in size. In contrast one female *G. holbrooki* could be responsible for the production of up to 9,000 young in one year. *G. holbrooki* at birth are much larger and robust than newly hatched larvae of *P. signifer*. Conceivably all four species of Australian pseudomugilid species that were studied in this thesis and *S. vermeilipinnis* (a vulnerable species found to cohabit with *G. holbrooki* in small ponds) could be disadvantaged by *G. holbrooki*.

It was suggested by Douglas *et al.*, (1994) that environmental degradation eliminates native fish allowing introduced species to replace them. Alternatively direct replacement of the native by the introduced species may occur. The results of the tank study (Chapter 4) would lend support for the latter hypothesis, as in the experimental ponds *G. holbrooki* were able to grow and reproduce at the expense of *P. signifer*. However the results of the pilot field study lend support to the former hypothesis as the field data did not show that *P. signifer* were reproductively compromised when found at sites with *G. holbrooki*. However abundances of *P. signifer* could be related to water quality parameters. Since *G. holbrooki* is most successful in disturbed habitats (Courtenay and Meffe, 1989) water bodies should be

managed to cause the least damage. *G. holbrooki* are replacing our native species as we are promoting unfavourable conditions for our native species by damming, diverting and channelling rivers. Many natural phenomena such as flooding have inhibited *Gambusia*'s success (Meffe and Snelson, 1989).

The widespread distribution of *G. holbrooki* and possible decline of several of our native species (*Ambassis*, *Chlamydogobius*, *Craterocephalus*, *Galaxias*, *Melanotaenia*, *Mogurnda*, *Pseudomugil*, *Retropinna* and *Scaturiginichthys*) (Wager and Jackson, 1993 Unmack and Paras, 1995) suggest that careful consideration should be made before other exotic fish species are released into Australian waterways. No fish should be introduced without public debate and independent scientific research and opinion (Tilzey, 1980). There should be reasons for the introduction, followed by a search for possible native fish to fulfil the requirements (Tilzey, 1980 and Courtenay and Meffe, 1989).

In summary.

This project has been successful in providing further understanding of the reproductive biology of the genus *Pseudomugil* and in particular *P. signifer*, in the field and under laboratory conditions. The study has also shown that the exotic species *G. holbrooki* is capable of having a significant adverse impact under laboratory conditions and that this impact is not simply the result of competition for food resources. Having carried out a pilot field study to determine the amount of sampling required, it would be of interest to carry out further research to confirm any adverse impacts of the introduced species on *P. signifer* in the field.

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APPENDIX I.
Histological techniques.

Full descriptions of procedures for embedding gonads in paraffin and for staining the resulting sections, employed in the studies described in Chapter 3. The procedure is as described in Culling, Allison and Barr (1985).

Protocol 1A: To embed gonads in paraffin for sectioning (Chapter 3).

Medium	%	Time
Ethyl Alcohol	70	5 hours
Ethyl Alcohol	85	20 minutes
Ethyl Alcohol	95	20 minutes
Ethyl Alcohol	100	20 minutes
New Ethyl Alcohol	100	20 minutes
New Ethyl Alcohol	100	20 minutes
Benzene	100	20 minutes
New Benzene	100	20 minutes
New Benzene	100	20 minutes
Paraplast		2 hours
New Paraplast		2 hours
New Paraplast		1 hour
Paraplast under vacuum		30 minutes
Embed in paraplast		

Protocol IB. To stain gonad sections on slides, (Chapter 3).

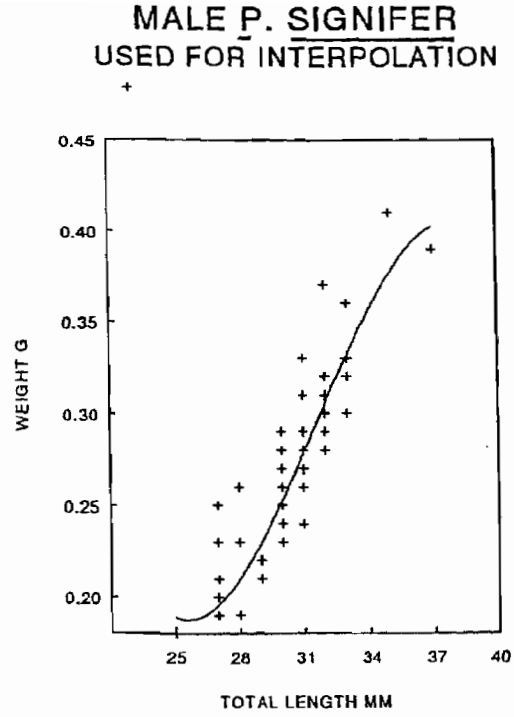
Medium	Time
Xylene	3-5 minutes
New xylene	3-5 minutes
Absolute Alcohol	1-3 minutes
New Absolute Alcohol	1-3 minutes
70% Alcohol	1-3 minutes
Wash in tap water	30 seconds
Haematoxylin	5-10 minutes
Wash in running water	1-2 minutes
Acid Alcohol	5-15 seconds
Rinse in running water	1-2 minutes
Scotts solution	2 minutes
Rinse in running water	1-2 minutes
Stain in eosin	1 minute
Rinse in running water	1-2 minutes
70% Alcohol	30 seconds
90% Alcohol	1 minute
Absolute Alcohol	1-3 minutes
New Absolute Alcohol	1-3 minutes
New Absolute Alcohol	1-3 minutes
Xylene	1-3 minutes
New Xylene	1-3 minutes
New Xylene	1-3 minutes
Mount in DPX	

APPENDIX II.

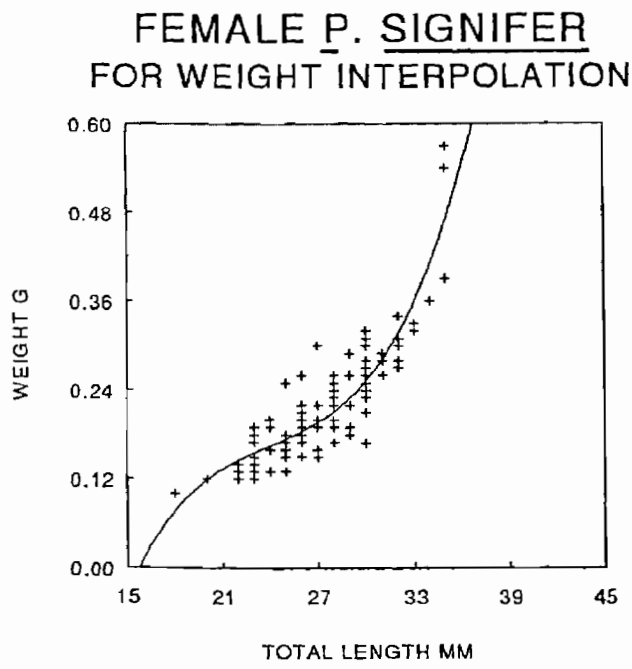
Interpolation graphs used for the determination of initial weights in *Pseudomugil signifer* and *Gambusia holbrooki*.

Initial weights for *Pseudomugil signifer* and *Gambusia holbrooki* in the tank studies described for Chapter 4 were interpolated from a weight curve derived from length/weight data, collected from the same population as those used in the experiments. This was done to avoid high handling mortalities which occurred when *P. signifer* were weighed prior to use in pilot experiments (75% of the twenty male and 78% of the twenty-seven female *P. signifer* died after weighing and before introduction to the tanks).

A



B

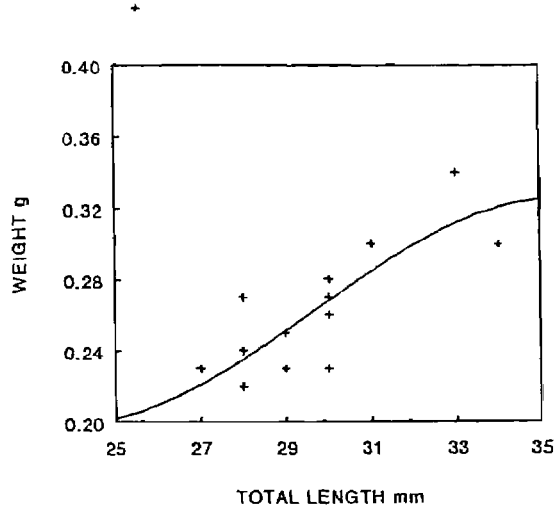


Total length/weight data for male (A) and female (B) *Pseudomugil signifer* collected from fish from the same population as those used in experiments in Chapter 4.

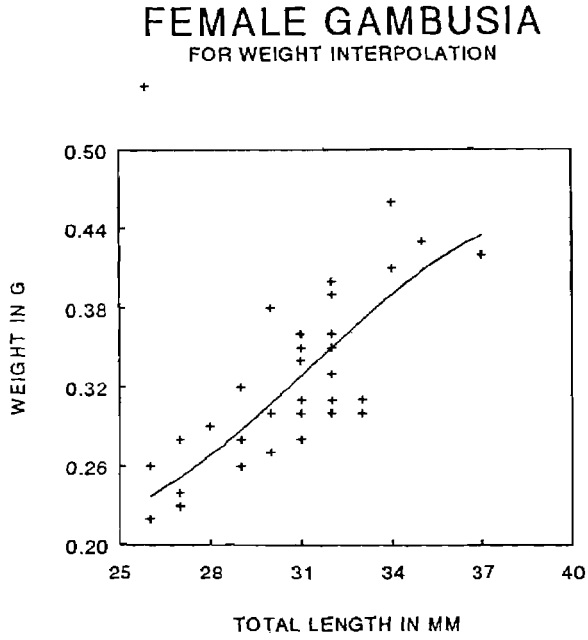
Fitted curve for the male is:-
Weight = $7.144 - 0.703x + 0.023x^2 - 0.0002x^3$
where x = total length and R = 0.879.

Fitted curve for the female is:-
Weight = $-2.217 + 0.279x - 0.011x^2 + 0.0002x^3$
where x = total length and R = 0.903.

A



B



Total length/weight data for male (A) and female (B) *Gambusia holbrooki* collected from fish from the same population as those used in experiments in Chapter 4.

Fitted curve for the male is:-

Weight = $4.421 - 0.454x + 0.016x^2 - 0.0002x^3$

where x = total length and R = 0.855.

Fitted curve for the female is:-

Weight = $2.293 - 0.295x + 0.010x^2 - 0.0001x^3$

where x = total length and R = 0.816.

APPENDIX III.

Publications arising from this thesis

1. Howe, E. (1987). Breeding behaviour, egg surface morphology and embryonic development in four Australian species of the genus Pseudomugil (Pisces: Melanotaeniidae).
Aust. J. Mar. Freshw. Res. 38, 885-895.

2. Howe, E. Howe, C. and Doyle, S. (1988). The surface of the eggs in blue-eyes *Pseudomugil* spp. **Fishes of Sahul 5(2)**, 205-216.

3. Howe, E. and Howe, C. (1991) Seasonal reproductive activity in two populations of the Pacific blue eye, *Pseudomugil signifer* Kner (Melanotaeniidae). **Fishes of Sahul 6(3)**, 268-276.