

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

EFFIE HELENA IRENE HOWE

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

University of Technology, Sydney Australia.

JUNE, 1995.

A



B



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

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

ACKNOWLEDGEMENTS.

I would like to thank Dr. R. Lim for accepting me as a student and for helpful discussion. I would also like to thank my co-supervisor, Associate Professor M. Burchett. I particularly wish to acknowledge her encouragement and patience during a long writing-up period.

The work contained in this thesis was made easier by the technical support at the University of Technology, Sydney. Thanks go to Ms. N. Richardson, Ms. B. Almond, Ms. S. Fenech and Mr P. Ralph who supported my laboratory work. Mr W. Hayes provided assistance in the analysis of nitrates in water. Associate Professor D. Cheng provided suggestions for the analysis of phytoplankton. Thanks also to Mr G. Hampshire from the University of Technology Library, Gore Hill, who provided me with many inter-library loans.

Both Ms. S. Doyle from the School of Biological Sciences, Macquarie University and Mr P. Jamieson from the University of Technology, Sydney provided me with expert assistance, on the use of the scanning electron microscope. Mr R. Oldfield from the School of Biological Sciences, Macquarie University provided expert photographic assistance during early work.

I would also like to acknowledge the support of Dr. W. Ivantsoff for his support in the early part of this research.

I would like to acknowledge the advice of members of staff of NSW State Fisheries, especially Dr. D. Pollard and Dr. J. Harris on the field component of the thesis.

I would like to acknowledge the editorial assistance and advice of Dr. R. McGee, Dr. D. Booth and Dr. V. Mawson. The late Emeritus Professor F. Mercer provided editorial assistance and encouragement for the manuscript on embryology.

I am grateful to Associate Professor D. Handelsman from the University of Sydney for advice on statistical methods.

The Australian Geographic Society kindly provided me with a small grant that enabled me to purchase photomicrography equipment.

Mr C. Taylor and Mr G. Lane from CSIRO Food Science and Technology provided assistance with the colour printing.

Thanks to my colleagues at work, in particular Dr. Don Barnett and Dr. Margaret Tyler for their moral support.

Without the advice, encouragement and field assistance of my husband, Chris this thesis would not have been written. Thanks also to my children Sean and Gregory, who have patiently waited for its completion.

TABLE OF CONTENTS.

Chapter 1.	1
General Introduction.	1
Studies in the biology and reproductive characteristics of <i>Pseudomugil signifer</i>	1
1.1 Context of project:- Australian freshwater fish fauna and the aims of the project.	1
1.2 Review of factors affecting growth and survival in <i>Pseudomugil</i>	4
1.2.1 Water quality parameters.	4
1.2.1.1 pH	4
1.2.1.2 Temperature.	4
1.2.1.3 Dissolved oxygen.	5
1.2.1.4 Biological oxygen demand (BOD).	5
1.2.1.5 Ammonia.	6
1.2.1.6 Nitrate.	8
1.2.1.7 Hardness.	8
1.2.1.8 Suspended sediment.	8
1.2.2 The genus <i>Pseudomugil</i>	9
1.2.2.1 Taxonomy.	9
1.2.2.2 Distribution.	10
1.2.2.3 Biology.	11
1.2.2.4 Ecology.	15
1.2.3 Exotics.	18
1.2.3.1 Exotic fish species.	19
1.2.3.2 Exotic fish in Australia.	20
1.2.3.3 <i>Gambusia holbrooki</i> in Australia.	22
1.2.4 Members of the genus <i>Gambusia</i> :- <i>Gambusia holbrooki</i> and <i>Gambusia affinis</i>	24
1.2.4.1 Taxonomy.	24
1.2.4.2 Distribution.	25
1.2.4.3 Description.	27
1.2.4.4 Ecology.	28
1.2.4.5 Reasons for the success of <i>G. holbrooki</i> and <i>G. affinis</i> following introduction.	32
1.3 Competition.	32
1.4 Objectives of present study.	33
1.4.1 Implications for management.	33
1.5 Experimental objectives.	34

Chapter 2.	37
Comparative study of reproductive biology of genus <i>Pseudomugil</i> (Pisces: Melanotaeniidae).	37
2.1 Introduction.	37
2.2 Materials and Methods.	39
2.2.1 Collection sites.	39
2.2.2 Collection of fish.	41
2.2.3 Acclimation and maintenance of stock fish.	41
2.2.4 Egg production and handling.	41
2.2.5 Surface structure of eggs.	42
2.2.6 Prespawning and spawning behaviour.	42
2.2.7 Embryonic development.	42
2.2.8 Larval development.	42
2.3 Results.	43
2.3.1 Structure of the egg.	43
2.3.1.1 Chorionic filaments and surface structure.	43
2.3.2 Prespawning and spawning behaviour.	50
2.3.3 Embryonic development.	53
2.3.3.1 Hatching.	59
2.3.4 Larval development.	59
2.3.4.1 Finfold.	59
2.3.4.2 Fins.	60
2.3.4.3 Myomeres.	61
2.3.4.4 Melanophores.	61
2.3.4.5 Squamation.	61
2.3.4.6 Secondary sexual characteristics.	61
2.4 Discussion.	62
2.4.1 Egg characteristics.	62
2.4.2 Is a saline environment necessary for breeding?	63
2.4.3 Review of <i>Pseudomugil</i> species.	63
Chapter 3.	68
Field study of reproductive activity in <i>P. signifer</i>	68
3.1 Introduction.	68
3.2 Materials and Methods.	69
3.2.1 Sites.	69
3.2.2 Fish collection.	72
3.2.3 Gonad examination - light microscopy.	73
3.2.4 Water parameters.	75
3.2.5 Rainfall.	75

3.3 Results.	77
3.3.1 Abundance.	77
3.3.2 Fish collection.	77
3.3.3 Standard length.	77
3.3.4 Gonads.	80
3.3.5 Water quality parameters.	85
3.3.6 Rainfall.	90
3.4 Discussion.	90
3.4.1 Abundance and reproductive condition.	90
3.4.2 Possible factors that might affect abundance.	91
3.4.3 Reproductive cycle.	92
3.4.4 Information obtained from different sites and laboratory study.	92
3.4.5 Factors that might affect growth and reproduction of <i>P. signifer</i>	92
Chapter 4.	95
Impact of <i>Gambusia holbrooki</i> on growth and reproduction of <i>Pseudomugil signifer</i> (experimental study).	95
4.1 Introduction.	95
4.2 Materials and Methods.	98
4.2.1 Tank Experiment I - The impact of <i>G. holbrooki</i> on the survival, growth and reproduction of <i>P. signifer</i>	98
4.2.1.1 Duration of experiment.	98
4.2.1.2 Tank set-up.	98
4.2.1.3 Water quality analysis.	99
4.2.1.4 Fish collection.	102
4.2.1.5 Fish measurement.	102
4.2.1.6 Ratio and stocking numbers.	102
4.2.1.7 Design.	103
4.2.1.8 Fish introduction and harvest.	104
4.2.2 Tank Experiment II - Impact of <i>G. holbrooki</i> on the fecundity of <i>P. signifer</i> , with and without augmented food supply.	104
4.2.2.1 Duration.	107
4.2.2.2 Tank set-up.	107
4.2.2.3 Water quality analysis.	107
4.2.2.4 Fish collection.	107
4.2.2.5 Fish measurement, stocking ratio and sex ratios.	107
4.2.2.6 Fish introduction and harvest.	107
4.2.2.7 Gonadosomatic index.	109
4.2.2.8 Statistical analysis.	109
4.2.3 Laboratory behavioural studies.	109
4.2.3.1 Behavioural observation I: Interactions of <i>P. signifer</i> housed with and without <i>G. holbrooki</i>	111
4.2.3.2 Behavioural observations II. Interactions between adult <i>P. signifer</i> and <i>G. holbrooki</i> and larval <i>P. signifer</i>	112

4.3 Results.	113
4.3.1. Tank experiment I - Impact of <i>G. holbrooki</i> on the survival, growth and reproduction of <i>P. signifer</i>	113
4.3.1.1 Water quality.	113
4.3.1.2 Fish parameter measurements: <i>P. signifer</i>	121
4.3.1.3 Summary of findings.	131
4.3.2. Tank experiment II - Impact of <i>G. holbrooki</i> on the fecundity of <i>P. signifer</i> , with and without augmented food supply.	134
4.3.2.1 Water quality.	134
4.3.2.2 Fish parameter measurements.	141
4.3.2.3 Synthesis of findings.	162
Juvenile <i>P. signifer</i> and <i>G. holbrooki</i>	162
4.3.3 Behavioural observations.	168
4.3.3.1 Behavioural observations I -interactions of <i>P. signifer</i> housed with and without <i>G. holbrooki</i>	168
4.3.3.2 Laboratory behavioural experiment II - interactions between adult <i>P. signifer</i> and <i>G. holbrooki</i> to larval <i>P. signifer</i>	170
4.4 Discussion.	170
4.4.1 Growth and behavioural patterns.	171
4.4.2 Water quality.	172
4.4.2.1 Temperature.	172
4.4.2.2 pH.	172
4.4.2.3 Dissolved oxygen.	173
4.4.2.4 BOD.	173
4.4.2.5 Ammonia, Nitrate and Hardness.	173
4.4.2.8 Invertebrates and phytoplankton.	174
4.4.3 Physiological mechanisms of growth inhibition.	174
4.4.4 Possible reasons for decline in reproductive activity of <i>P. signifer</i> when housed with <i>G. holbrooki</i>	176
4.4.4.1 Interruption of normal behaviour patterns.	177
4.4.4.2 Endocrine response.	177
4.4.4.3 Physiological stress.	178
4.4.4.4 Stocking density.	179
4.4.4.5 Territoriality.	180
4.4.5 Food as a limiting resource in the test tank experiments of <i>P. signifer</i> when housed with <i>G. holbrooki</i>	180
4.4.6 Effect of food restriction on <i>G. holbrooki</i>	181
4.4.7 Possible reasons for the difference in response of the two species to supplementary feeding.	181
4.4.8 How exotic species may affect native species.	182
4.4.9 Replacement mechanisms.	182
4.4.9.1 Parasites and disease.	182
4.4.9.2 Hybridisation.	182
4.4.9.3 Competition.	183
4.4.9.4 Predation.	183
4.4.10 Future research and concluding remarks.	184

Chapter 5.	187
Pilot Study: Field investigation of effects of habitat conditions and presence of <i>Gambusia holbrooki</i> on <i>Pseudomugil signifer</i>	187
5.1 Introduction.	187
5.2 Materials and Methods.	188
5.2.1 Site selection.	188
5.2.2 Site descriptions:- Lane Cove and Narrabeen.	189
5.2.2.1 Homebush Bay.	195
5.2.3 Rainfall.	199
5.2.4 Water quality.	202
5.2.5 Sampling.	203
5.2.6 Statistical analysis.	204
5.3 Results.	206
5.3.1 Rainfall.	206
5.3.2 Water quality.	206
5.3.3 Measurements of fish.	212
5.3.3.1 Abundances (Fig. 5.13).	212
5.3.3.2 Total and standard length and weight (Fig. 5.14 A, B and C).	214
5.3.3.3 Testis weight (Fig. 5.15A).	216
5.3.3.4 Ovarian weight.	216
5.3.3.5 Gonadosomatic index (GSI) of male <i>P. signifer</i>	220
5.3.3.6 Gonadosomatic index and fecundity of female <i>P.</i> <i>signifer</i> (Fig. 5.17 B and C).	220
5.3.3.7 Comparison of fecundity in tank experiment II (Chapter 4) and the field study.	222
5.3.4 Caudal fin.	222
5.4 Discussion.	223
5.4.1 Water quality.	225
5.4.2 Rainfall.	228
5.4.3 Abundance.	228
Power Analysis	230
5.4.4 Disturbed areas.	230
5.4.5 The impact of exotic introductions.	231
5.4.6 Impact of <i>Gambusia</i> on various species of fish.	232
5.4.7 Potential for restricted Pseudomugilidae.	233
Chapter 6.	234
General Discussion.	234
6.1. Background.	234
6.2 Major findings.	234
6.3 Strategies used by <i>P. signifer</i> and <i>G. holbrooki</i>	239
6.3.1 Reproduction.	239

6.3.2 Comparative size of the two species and growth rates.	241
6.3.3 Territoriality.	242
6.3.4 Aggression.	242
6.3.5 Predation and cannibalism.	243
6.3.6 Fitness of the species in relation to their evolutionary development.	244
6.3.7 Genetic diversity.	245
6.3.8 Geographic isolation of Australia.	246
6.3.9 Summary of possible survival advantages <i>G. holbrooki</i> has over <i>P. signifer</i>	248
6.4 Need for further research.	249
6.5 Management.	251
In summary.	253
References	254
Appendix I - Histological Methods (Chapter 2).	282
Appendix II - Interpolation of length/weight curves in <i>P. signifer</i> and <i>G.</i> <i>holbrooki</i> (Chapter 4).	284
Appendix III - Publications arising from this thesis	287

"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)

LIST OF FIGURES.

Figure 1.1 Drainage division boundaries on the Australian continent (Merrick and Schmida, 1984) and distribution of <i>Pseudomugil</i> species	2
Figure 1.2 Distribution range of <i>Gambusia holbrooki</i> in Australia.	26
Figure 2.1 Location of the sites where <i>Pseudomugil signifer</i> , <i>Pseudomugil mellis</i> , <i>Pseudomugil gertrudae</i> and <i>Pseudomugil tenellus</i> were sampled.	40
Figure 2.2 Scanning electron microscope photomicrographs of eggs	44
Figure 2.3 Scanning electron microscope photomicrographs of pattern of filaments on eggs	45
Figure 2.4 Scanning electron microscope photomicrographs of a single filament .	46
Figure 2.5 Scanning electron microscope photomicrographs of surface sculpturing	47
Figure 2.6 Scanning electron microscope photomicrographs of the micropyles . .	48
Figure 2.7 Embryonic development	54
Figure 2.8 Time course of embryological development	57
Figure 3.1 Location of sampling sites	70
Figure 3.2 Stages of testicular development	76
Figure 3.3 Standard lengths	78
Figure 3.4 Weight of fixed ovaries	81
Figure 3.5 Ovarian photomicrographs	83
Figure 3.6 Salinity of water at field sites	87
Figure 3.7 Average monthly rainfall at Sydney (A) and Newport (B)	88
Figure 3.8 Hours of daylight and water temperatures throughout the year.	89
Figure 4.1 Experiment I. Diagrammatic representation of the random allocation of treatments.	105
Figure 4.2 The eight tanks used in Experiment I prior to harvest	106
Figure 4.3. Establishment of the sixteen tanks used in Tank Experiment II	108

Figure 4.4 Experiment I. pH (A) and temperature (B)	114
Figure 4.5 Experiment I. Dissolved oxygen concentrations (A) and biological oxygen demand (B)	116
Figure 4.6 Experiment I. Un-ionised ammonia concentrations (A) and nitrate concentrations (B)	118
Figure 4.7 Experiment I. Hardness levels	119
Figure 4.8 Experiment I. Mean number of individual male (A) and female (B) <i>Pseudomugil signifer</i>	122
Figure 4.9 Experiment I. Total length (A) and standard length (B) of individuals of male <i>Pseudomugil signifer</i>	123
Figure 4.10 Experiment I. Total length (A) and standard length (B) of individuals of female <i>Pseudomugil signifer</i>	124
Figure 4.11 Experiment I. Weights of male (A) and female (B) individuals of <i>Pseudomugil signifer</i>	125
Figure 4.12 Experiment I. Mean number of individuals male (A) and female (B) <i>Gambusia holbrooki</i>	127
Figure 4.13 Experiment I. Total length (A) and standard length (B) of individual male <i>Gambusia holbrooki</i>	128
Figure 4.14 Experiment I. Total length (A) and standard length (B) of individual female <i>Gambusia holbrooki</i>	129
Figure 4.15 Experiment I. Weight of male (A) and female (B) individuals of <i>Gambusia holbrooki</i>	130
Figure 4.16 Experiment I. Mean number of juveniles of <i>Pseudomugil signifer</i> and <i>Gambusia holbrooki</i> at harvest	132
Figure 4.17 Experiment II. pH (A) and temperature (B)	135
Figure 4.18 Experiment II. Dissolved oxygen concentrations (A) and biological oxygen demand (B)	137
Figure 4.19 Experiment II. Un-ionised ammonia (A) and nitrate (B) concentrations	138
Figure 4.20 Experiment II. Hardness levels	139
Figure 4.21 Experiment II. Mean number of individuals male (A) and female (B) <i>Pseudomugil signifer</i>	142

Figure 4.22 Experiment II. Total length (A) and standard length (B) of individuals of male <i>Pseudomugil signifer</i>	144
Figure 4.23 Experiment II. Total length (A) and standard length (B) of individuals of female <i>Pseudomugil signifer</i>	145
Figure 4.24 Experiment II. Weight of male (A) and female (B) individuals of <i>Pseudomugil signifer</i>	147
Figure 4.25 Experiment II. The ovary of <i>Pseudomugil signifer</i> at harvest.	148
Figure 4.26 Experiment II. Fecundity (A) and ovarian weight (B) of female <i>Pseudomugil signifer</i>	149
Figure 4.27 Fecundity plotted against body weight of <i>Pseudomugil signifer</i> at harvest	150
Figure 4.28 Ovarian weight plotted against body weight of <i>Pseudomugil signifer</i> at harvest	151
Figure 4.29 Experiment II. Mean testis weight of male <i>Pseudomugil signifer</i>	153
Figure 4.30 Experiment II. Mean gonadosomatic index (GSI) of male (A) and female (B) individuals of <i>Pseudomugil signifer</i>	154
Figure 4.31 Experiment II. Numbers of individual male (A) and female (B) <i>Gambusia holbrooki</i>	155
Figure 4.32 Experiment II. Total length (A) and standard length (B) of individuals of male <i>Gambusia holbrooki</i>	157
Figure 4.33 Experiment II. Total length (A) and standard length (B) of individuals of female <i>Gambusia holbrooki</i>	158
Figure 4.34 Experiment II. Weight of male (A) and female (B) individuals of <i>Gambusia holbrooki</i>	159
Figure 4.35 Experiment II. Fecundity of <i>Gambusia holbrooki</i>	160
Figure 4.36 Experiment II. Weight of male testis (A) and female ovaries (B) of <i>Gambusia holbrooki</i>	161
Figure 4.37 Experiment II. Gonadosomatic index in male and female <i>Gambusia holbrooki</i>	163
Figure 4.38 Experiment II. Mean number of individuals of juvenile <i>Gambusia holbrooki</i>	167

Figure 4.39 Experiment II. Intact and damaged caudal fins of <i>Pseudomugil signifer</i>	169
Figure 5.1 Location of the four sampling sites in the Sydney area	190
Figure 5.2 Location of the eight stations used for sampling <i>Pseudomugil signifer</i> at the Lane Cove River site	191
Figure 5.3 Two sampling stations at the Lane Cove site	192
Figure 5.4 Location of the eight stations used for sampling <i>Pseudomugil signifer</i> and <i>Gambusia holbrooki</i> at the Narrabeen site	193
Figure 5.5 Two sampling stations at the Narrabeen site	194
Figure 5.6 Location of the eight stations used for sampling <i>Pseudomugil signifer</i> and <i>Gambusia holbrooki</i> at the Homebush Bay site	196
Figure 5.7 Saline and freshwater sampling stations at the Homebush Bay site	197
Figure 5.8 Mangrove stations at the Homebush Bay site	198
Figure 5.9 Location of the eight stations used for sampling <i>Pseudomugil signifer</i> at the Mooney Mooney Creek site	200
Figure 5.10 Two sampling stations at the Mooney Mooney Creek site	201
Figure 5.11 Temperature (A), pH (B) and salinity (C) at the four sampling sites	209
Figure 5.12 Turbidity (A), un-ionised ammonia (B) and dissolved oxygen (C) at the four sampling sites	211
Figure 5.13 The total number of <i>Pseudomugil signifer</i> and <i>Gambusia holbrooki</i> (A) and average number of male and female <i>Pseudomugil signifer</i> (B) at the four sampling sites	213
Figure 5.14 Total lengths (A), standard lengths (B) and weights (C) of male (M) and female (F) <i>Pseudomugil signifer</i> at the four sampling sites	215
Figure 5.15 Average testis weight (A) and ovarian weight (B) of <i>Pseudomugil signifer</i> at the four sampling sites	217
Figure 5.16 Ovarian weight of <i>Pseudomugil signifer</i> plotted against body weight at harvest from the four sampling sites	218
Figure 5.17 Average gonadosomatic index (GSI) of male (A), female (B) and average fecundity (C) of <i>Pseudomugil signifer</i>	219

Figure 5.18 Fecundity of <i>Pseudomugil signifer</i> plotted against body weight at harvest from the four sampling sites	221
Figure 6.1 Schematic sequence of the effects of environmental stress on estuarine animals	237

LIST OF TABLES.

Table 1.1 Species of Pseudomugilidae occurring in Australia and Papua New Guinea.	12
Table 2.1. Collection sites for species of <i>Pseudomugil</i>	39
Table 2.2 Comparison of the surface filaments of the eggs of <i>P. signifer</i> with <i>P. mellis</i> , <i>P. tenellus</i> and <i>P. gertrudae</i>	49
Table 2.3 Some characteristics of the micropyle in <i>Pseudomugil</i> species.	51
Table 2.4 Spawning colouration in males of four <i>Pseudomugil</i> species.	52
Table 2.5. Comparison of the eggs of <i>P. signifer</i> with <i>P. mellis</i> , <i>P. tenellus</i> and <i>P. gertrudae</i>	58
Table 2.6. Minimum lengths of larvae at which some major features were first observed	60
Table 3.1. Classification of oocytes in <i>P. signifer</i> ovaries.	74
Table 3.2. Numbers of sexed <i>Pseudomugil signifer</i> caught.	79
Table 3.3 Numbers of other fish species caught at Lane Cove and Deep Creek	79
Table 3.4. Ovarian development in <i>P. signifer</i> , from February 1985 to January 1986.	82
Table 3.5. Fecundity	84
Table 3.6. Development of testes from February 1985-January 1986 in <i>P. signifer</i> collected from Deep Creek.	86
Table 3.7. Development of testes from February 1985-January 1986 in <i>P. signifer</i> collected from Lane Cove River.	86
Table 4.1 Numbers of male and female <i>P. signifer</i> caught at a variety of sites .	103
Table 4.2. Methods used for measurements performed on fish	110
Table 4.3. Experiment I. Abundance of invertebrates	120
Table 4.4. Experiment I. Abundance of phytoplankton	120

Table 4.5. Experiment I. Summary of analysis	133
Table 4.6 Experiment II. Abundance of invertebrates	140
Table 4.7 Experiment II. Abundance of phytoplankton	141
Table 4.8 Experiment II. A summary of changes in growth parameters	164
Table 4.9 Experiment II. Summary of tests on reproductive parameters at harvest.	166
Table 4.10 Observations of <i>P. signifer</i> with and without <i>G. holbrooki</i>	168
Table 5.1 Rainfall during 1993 at Sydney and Newport	202
Table 5.2 Dates of sampling at the four sites.	203
Table 5.3 Mean water quality parameters at the four sites	206
Table 5.4 Water quality parameters at individual stations at the Homebush Bay site.	207
Table 5.5 Summary of significant analyses.	223

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.