The reproductive biology and induced spawning of striped trumpeter, *Latris lineata.*

WAYNE HUTCHINSON
B. Ag. Sc., Grad. Dip. Ed. (Sec.),
Grad. Dip. App. Sc. (Aqua.)

Submitted in fulfilment of the requirements for the degree of Master of Applied Science in Aquaculture by Research.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution. To the best of my knowledge and belief this thesis contains no material previously published by another person, except where due reference has been made in the text of the thesis.

Wayne Hutchinson.

Wayne Hutchinson.

TITLE.

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ABSTRACT.

Sampling of *Latris lineata* (n= 334) was conducted with commercial fishers on the east coast of Tasmania between May 1990 and May 1992. Relationships between fork length and weight, and age and fork length are presented for *Latris lineata*. Macroscopic descriptions of the stages of gonad maturation for male and female *Latris lineata* are verified from histological studies. A gonad index (GI = gonad weight (g)/fork length (cm) x 10^5) is used to show that the spawning season of *Latris lineata* extends from September to October. A critical GI value of approximately 25 is proposed to distinguish female *Latris lineata* in advanced stages oocyte development prior to spawning. Analysis of frequency distributions of the diameters of developing oocytes shows that three distinct batches of developing oocytes can be distinguished in the ovaries of *Latris lineata* approaching spawning. This information indicates that female *Latris lineata* spawns multiple batches of eggs, which develop in a group synchronous manner. Female *Latris lineata* mature earlier (5 years; FL = 43.8 cm) than males (8 years; FL = 53.4 cm).

A study in 1990 suggested that intra-peritoneal injection of 0.5 ml/kg b.w. of Ovaprim® (Salmonid gonadotropin releasing hormone (sGnRH)with domperidone) could induce ovulation in *Latris lineata*. An investigation in 1991 compared interperitoneal (i.p) injection of Ovaprim ^{R} with i.p. injection of lutenizing hormone releasing hormone analogue (LHRHa) at dosages of 10μg/kg b.w., 25μg/kg b.w., and 50μg/kg b.w. No significant difference ($P > 0.05$) between treatments was shown. Females with initial oocyte diameters of 600μm or greater were induced to complete final oocyte maturation. Two experiments conducted in 1992 investigated the effect of monthly (July-September) intramuscular implantation of cholesterol (90%) and copha (10%) pellets containing LHRHa and 17α-methyltestosterone (17α-MT) on circulating levels of 17β-estradiol and testosterone. One experiment found no significant effect ($P > 0.05$) of a high and low dose LHRHa+17α-MT implant regime, on mean oocyte diameter. Only one hormone implanted fish ovulated a batch of eggs in this investigation. No significant effect ($P > 0.05$) on mean serum 17β-estradiol and testosterone due to implant treatment dosage was shown. Mean serum levels of 17β-estradiol showed no significant ($P > 0.05$) change at 24 hr following implanting, for any treatment, at any month. For high dose hormone treatment fish, mean serum testosterone had decreased significantly ($P < 0.05$) at 24 hr following the July implant, while mean testostoterone of control fish showed a significant increase ($P < 0.05$) at this time. In the second experiment, 2 of 7 mature female *Latris lineata* implanted with the high dose LHRHa+17α-MT pellet formulation were induced to ovulate. One fish ovulated 11 times and the other ovulated 9 times, mostly at 3 day intervals. The first of these fish ovulated a batch of eggs 21 days before the first ovulation by a control fish. Both control fish released one egg batch spontaneously. No significantly ($P > 0.05$) change in either mean oocyte diameter or mean serum levels of 17β-estradiol or testosterone were found between hormone implanted and control fish, at any month. Mean serum 17β-estradiol was significantly ($P < 0.05$) higher in the 2 implanted fish, than control fish, at the time of the first (July) and second (August) implants. A significant ($P < 0.05$) decrease in mean serum testosterone of hormone treated fish was recorded at 144 hr after implanting in July, while a significant ($P < 0.05$) increase in this steroid was recorded at 144 hr following the August implant.

ACKNOWLEDGMENTS.

Considerable assistance has been provided to me during the research and compilation components of this thesis. The topic undertaken started as a postgraduate student project and would not have been continued without the initial endeavours of Mr. Karl Van Drunen and Mr. John Mikolajunas. From the outset, the support and assistance provided by all of the academic and technical staff at the University of Tasmania at Launceston, Key Centre for Aquaculture has been unwavering. Particular thanks go to Mr. Piers Hart for many a lively discussion and keen interest, Professor Nigel Forteath for his continual support, Dr. Leong Wee for his supervisory contributions and encouragement, Dr. John Purser for his general enthusiasm for the project, Dr. Barry Munday for diagnosis of fish health concerns and Professor Andrew Osborne for his constructive critical input during compilation of this thesis.

I would also like to acknowledge the assistance provided by the technical staff of the Key Centre for Aquaculture in particular Mr. Detlef Planko for his constant support in fish husbandry matters, Mr. Richard MacDonald for acquiring among other things, tank components, and Mr. Matthew Vandermolen and Mr. Mark Hilder for keeping the broodfish well fed and the holding system operational in my times of absence. In this regard I also acknowledge Mr. Ian Renken at the Australian Maritime College for making a steady supply of fresh feed for the broodfish available at no cost.

The reproductive information gathered from wild-caught fish in this thesis could not have been achieved without the generous and unassuming contributions of two commercial fishermen, Mr Noel Harper and Mr. Eddy Freeman, both of whom offered their assistance enthusiastically, at any possible opportunity. The contributions of the Tasmanian Department of Sea Fisheries are also acknowledged. In particular I thank Mr. Lance Searle for allowing the use of fish and facilities, and Mr. Leigh Oates for his assistance with the running of the 1991 and 1992 spawning induction trials.

Other people who provided assistance to this research project during its compilation in Adelaide include Mr. Alan Gilmour at the Queen Elizabeth Hospital who conducted radioimmunoassays of duplicate serum samples, and Ms. Janine Baker of the South Australian Research and Development Institute who provided guidance in the processing of otolith samples. Lastly and most importantly I acknowledge the personal support of Ms. Lynnette Mawby who has been an inexhaustible source of positive inspiration throughout the time it has taken to compile this thesis.

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INTRODUCTION.

Striped trumpeter, *Latris lineata*, is a highly regarded native fish species which is being investigated as a potential candidate to diversify marine fish aquaculture operations in southern Australia. The objective of this study was to document the reproductive development of wild caught *Latris lineata* and use this information as a foundation upon which to develop methods to control egg production from captive broodstock.

This thesis reviews the current literature relating to *Latris lineata* (Chapter 1). General biological information compiled from wild fish captured during this study adds to this background knowledge (Chapter 2). A review of the developmental events and physiology of reproduction in teleosts is presented (Chapter 3) as a prelude to a detailed investigation of the reproductive biology of *Latris lineata* (Chapter 4). Specifically the stages of reproductive development for male and female *Latris lineata* are documented from macroscopic and histological studies. Age and size at first maturity for both sexes of *Latris lineata*, are presented together with a description of the annual pattern of reproductive development compiled from gonad index data. Frequency distribution of oocyte diameters from female fish at different stages of reproductive development is used to propose a mode of spawning for this species.

Reproductive endocrinology of teleosts and the application of this information to control the spawning of teleosts is reviewed (Chapter 5). Methods to induce spawning were investigated during this study in order to develop a method which would provide controlled egg production from captive *Latris lineata* broodstock. These investigations were conducted over three successive spawning seasons (1990 - 1992). A description of each of these investigations and the results obtained are presented (Chapter 6), together with a discussion of the significance of the findings of this thesis (Chapter 7).

1 BACKGROUND INFORMATION RELATING TO STRIPED TRUMPETER, *Latris lineata*.

Information directly relevant to striped trumpeter is scarce with no scientific or fisheries studies on this species being reported. As a result of this situation the knowledge presented has been collected from a combination of current and historical fisheries publications containing minor references to this species.

1.1 Classification.

The classification of striped trumpeter, *Latris lineata*, adopted for this study is that of Last *et al.*, 1983 .

KINGDOM	ANIMALIA
PHYLUM	CHORDATA
CLASS	OSTEICHTHYES
ORDER	PERCIFORMES
FAMILY	Latridae
GENUS and SPECIES	*Latris lineata.*
COMMON NAME	Striped trumpeter

1.2 Common names.

Striped trumpeter, common trumpeter, Hobart trumpeter, Hobart-town trumpeter, real trumpeter, stripy, Tasmanian trumpeter, trumpeter, kohikohi (Johnston, 1890; Ayling and Cox, 1982; Last *et al.*, 1983; Francis, 1988).

1.3 Naming.

Striped trumpeter was originally described by Bloch and Schneider (Syst. Ichth. 1801 p 342) as *Cichla lineata* however *Latris hecateia* was the appellation adopted by Richardson (Tr. Zool. Soc. iii, 1842, p. 106) when describing a collection of fish formed at Port Arthur in southern Tasmania by the Assistant Commissionary General, T.J Lempriere Esq; under the direction of the Lieutenant Governor, his excellency Sir John Franklin (McCulloch, 1921). Early descriptions by Hector (1872), Johnston (1882, 1890) and Sherrin (1886) retain the name *Latris hecateia*, however M^{c}Culloch (1921) refers to the species as *Latris lineata* in his checklist of fish and fish-like animals of New South Wales, suggesting the change in nomenclature occurred within this period.

1.4 Physical description.

Some variation in structural descriptions has occurred between the reports from different authors presented in table 1.1; mostly in regard to the number of dorsal spines and rays, anal rays and the number of scales of the lateral line. This variation could be due to differences within populations taken from different geographical locations and differences in the morphology of individual fish used for examination.

Table 1.1 Comparison of structural features used for identification of *Latris lineata*.

AUTHOR	DATE	DORSAL FINS		ANAL FIN		PECTORAL	SCALES
		Spiny	Soft	Spines	Rays	FIN (rays)	ON LAT. LINE
Richardson	1842	18	36	3	27	-	-
Gunther	1864	17	1*.36	3	27	-	110
Johnston	1890	17	1*.36	3	28-30	-	100
Parrott	1959	18	35-36	3	27	-	-
Ayling & Cox	1982	18	35	3	32	-	114
Last *et al.*	1983	18	34-36	3	31-32	18	114
			* Spine				

1.4.1 Size.

The maximum size to which striped trumpeter grow is variously reported as presented in Table 1.2.

Table 1.2 Maximum lengths and weights reported for *Latris lineata*.

AUTHOR.	YEAR.	LOCATION.	LENGTH.	WEIGHT.
McCulloch	1921	N.S.W.	40 inches (102 cm)	-
Roughly	1951	Australia	48 inches (102 cm)	60 lb (27 kg)
Graham	1953	New Zealand	42 inches (107 cm)	60 lb (27 kg)
Parrott	1959	Aust./N.Z	48 inches (122 cm)	60 lb (27 kg)
Ayling & Cox	1982	New Zealand	100 cm	-
Last *et al.*	1983	Tasmania	120 cm	25 kg
Paul	1986	New Zealand	100 cm	-
Francis	1988	New Zealand	110 cm	-

1.4.2 Appearance.

The appearance of striped trumpeter (Figure 1.1) is unmistakable. This species has an elongate, compressed body marked with three prominent dark greenish-bronze longitudinal stripes along the upper body on each side, which extend to the base of the caudal fin, and a lower, more diffuse stripe on each flank. The name *lineata* refers to these stripes (Graham, 1953) which degenerate anteriorly into an irregular pattern of dark blotches covering the forehead and cheeks, and which reach the dorsal margin of the mouth. The remainder of the body is silvery white with a yellow tinge. Fins are conspicuous by their yellow colouration with the dorsal fin marked by dark blotches.

The caudal fin is forked and the pectoral fins are large and fan shaped. The dorsal fin is deeply notched and a scaly groove is present for reception of the spinous dorsal fin, which usually remains retracted. Striped trumpeter have a slightly protrusible mouth with bands of small villiform teeth lining the jaws and a small circular patch of these teeth on the vomer. In the posterior of the mouth, paired pharangeal pads are found. Scales are cycloid.

1.5 Distribution.

The distribution of striped trumpeter (Figure 1.2) extends along the southern coastline of Australia, being found in Tasmania, Victoria, New South Wales, South Australia (McCulloch, 1921; Roughly, 1951; Last *et al.*, 1983). Striped trumpeter is also a common fish in the southern waters of New Zealand being most abundant around the Otago Peninsula, Stewart Island (Parrott, 1957) and Kaikoura Peninsula (Hector, 1872; Doak, 1972). Although once quite common in commercial catches from the Cook Strait area (Doak, 1972) it is now rare north of Cook Strait (Francis, 1988) and is only occasionally found as far north as the Bay of Plenty on the north island of New Zealand (Ayling and Cox, 1982; Paul, 1986). Striped trumpeter is also found in the waters around the Snares Islands, Chatham Islands and Auckland Islands off New Zealand (Francis, 1988).

Within Australia striped trumpeter are more common in more southerly locations being most abundant in Tasmania (Parrott, 1959). In other Australian states this species is less common and it seems that the northern limit to its geographical range is restricted to the far south coast of New South Wales where it is found occasionally in considerable numbers at a location ten miles east of Bermagui appropriately named Trumpeter reef (Roughly, 1951; Parrott, 1959). It is reported that this species also occurs at some islands in the south Atlantic and Indian Oceans (Paul, 1986). All of

Figure 1.1 Striped trumpeter, *Latris lineata*.

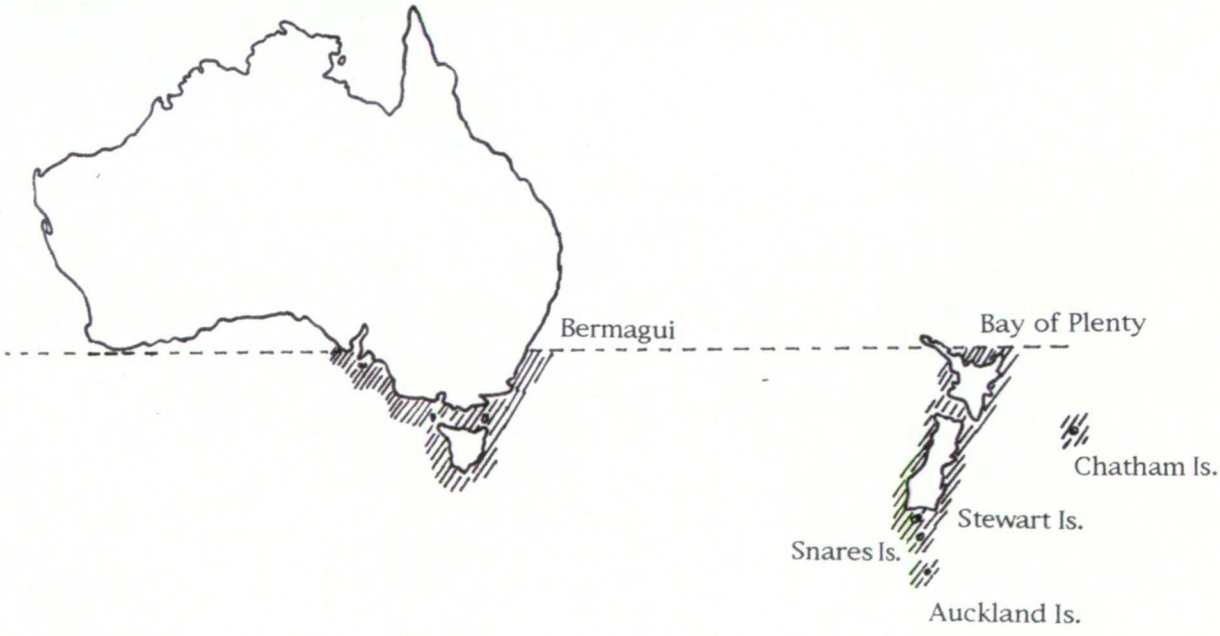


Figure 1.2 Distribution of striped trumpeter, *Latris lineata*, in Australian and New Zealand waters.

these reports suggest that *Latris lineata* is restricted in its' northerly distribution to coastal waters below approximately latitude 36^0 south.

1.6 Habitat.

Striped trumpeter inhabits inshore and off-shore, rocky reefs (Sherrin, 1886; Johnston, 1890; Roughly, 1951; Graham, 1953; Parrott, 1959; Last *et al.*, 1983; Paul, 1986). In the past fishermen would locate potential striped trumpeter grounds by testing the location "with heavy sinkers shod with grease. Any traces of coral were regarded as favourable indications that trumpeter were likely to be present" (Graham, 1953). Such locations are found in waters between 10-70 fathoms (20-140 m) (Graham, 1953; Ayling and Cox, 1982), although Parrott (1959) and Last *et al.* (1983) state that striped trumpeter can be found to a depth of 150 fathoms (300 m). Parrott (1957) quotes G.M. Thomson (unsighted) "this fish, as taken in Otago waters and sold in Dunedin fish-shops, is usually less than a foot long but quite recently (August or September 1912) very large specimens nearly three feet in length have been taken from 90 to 150 fathoms in two localities off Cape Saunders and Taiaroa Heads in large numbers". Commercial fishermen assisting in this study worked grounds off the east coast of Tasmania between 70-90 fathoms (140-180 m).

Several New Zealand authors are of the opinion that striped trumpeter move inshore during the summer and migrate to deeper off-shore reefs in winter. (Graham, 1953; Parrott, 1957; Ayling and Cox, 1982). No reference to such seasonal movement has been reported in Australian populations. It is apparent that Striped trumpeter are local in their occurrence remaining continually on certain patches of feeding ground (Hector, 1872; Parrott, 1957). Although striped trumpeter are a schooling fish, solitary fish are frequently observed. Large aggregations of striped trumpeter are found in the vicinity of deep off-shore reefs accounting for the reports of large hauls

of these fish being taken when such locations are found (Hector, 1872; Doak, 1972; Francis, 1988).

1.7 Feeding.

Striped trumpeter are carnivorous on benthic animals. Graham (1953) reports that in New Zealand striped trumpeter feeds on "pilchards, sprats, short-snouted pipe fish, long-snouted pipe fish, seahorse, octopus, squid, two species of mussels, the well known swimming crab, and whale feed (*Munidagregaria*) both in the swimming and bottom living forms. Broken shell and seaweed have also been taken from their stomachs." Francis (1988) confirms that this species has been seen feeding on large planktonic swarms of crustaceans (whale feed). Parrott (1957), Ayling and Cox (1982), and Paul (1986) agree that the prey of striped trumpeter includes octopus, squid, crabs, shrimps and small fishes Observations of stomach contents exposed while gonad sampling during this study confirm this description of the feeds taken by striped trumpeter in Tasmania. Thus this species is best described as an opportunistic feeder, accepting a wide variety of prey items available from the ecosystem of the reefs it inhabits.

1.8 Reproduction.

In New Zealand striped trumpeter is known to be a winter-spawning species (Sherrin, 1886; Parrott, 1957; Graham, 1953; Ayling and Cox, 1982; Francis, 1988). Graham (1953) claims that the breeding period of striped trumpeter was not known until July 1933, however Sherrin (1886) gives a good account of the spawning season of this species. This author, quotes information received from a fisherman, Mr. Rush, who stated that " the coral-reef trumpeter swim in large schools. They spawn in deep water in June, July and August. I have never seen mature ova in a fish under 15 lb (6.8 kg)". A Mr. Smith is also quoted as informing this author that he had caught fish full

of spawn in deep water, and had observed the milt running from them in May and June. The spawning season of striped trumpeter has not been documented in Australia however observations taken throughout the course of this study show that along the east coast of Tasmania this species spawns in spring, predominantly in September and October.

Graham (1953) reports the fecundity of striped trumpeter. However, although two methods were used, no description of either method used to determine values presented in Table 1.3 is provided.

Table 1.3 Fecundity of *Latris lineata* from Graham (1953).

CLEANED WEIGHT	ORIGINAL WEIGHT	NUMBER OF EGGS	FECUNDITY (Eggs/kg original wt.)
25 lb. (11.4 kg)	33 lb. (15 kg)	12,000,000	800,000
20 lb. (9.1 kg)	26 lb. (12 kg)*	10,000,000	833,333

* figure deduced from cleaned weight provided.

1.9 Life cycle.

It is generally accepted that immature striped trumpeter inhabit inshore reefs associated with bays and estuaries (Sherrin, 1886; Roughly, 1951). Sherrin (1886) reported that two varieties of trumpeter were brought to market in New Zealand. One type were refered to as "school fish" caught over a rocky bottom in shallow water near shore. These fish were small, immature and rarely exceeded 6-7 lbs (2.7-3.2 kg). The other type was larger, usually 15-20 lb (6.8-9.1 kg), caught over deep coral reefs and was usually full of roe or milt. This situation is also reported by Parrott (1959) who stated that commercial fishermen catch trumpeter of two size groups; one group being about 12 inches (30 cm) while the other group is about 24 inches (60 cm) in length. Last *et*

al (1983) states that juveniles up to 2.0 kg were once common in shallow waters of Tasmania but are now only found in more remote areas. The conclusion drawn by Sherrin (1886) is that following spawning in deep water, the young fry approach the shallower, rocky bottom nearer shore were they grow to the school fish type which migrates back to the deep coral reefs as they approach maturity. Graham (1953) suggests that spawning occurs well below 25 fathoms.

No reports specific to the distribution of larval and early life stages of have been undertaken. Whitley (1941) postulates that the little fishes known as "paper fish" and "silvery threadfin", found in south-eastern Australian and New Zealand waters, are the young of morwongs and trumpeters. Examination of the illustration of a juvenile silver (bastard) trumpeter *Latridopsis forsteri* specimen from the Tamar river shown in Figure 1.3 supports this conclusion in regard to this species.

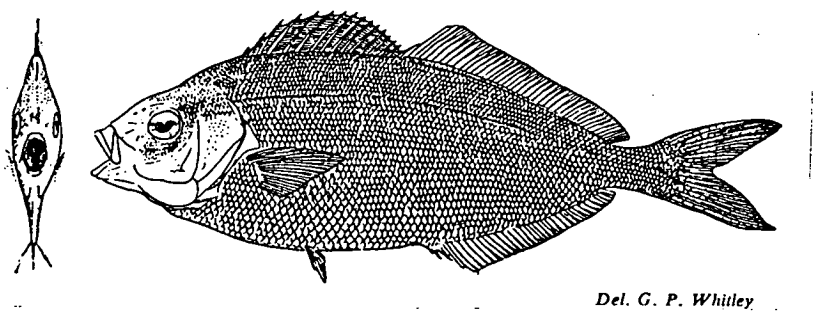


Figure 1.3 Juvenile Bastard (Silver) trumpeter *Latridopsis forsteri*, known as Paper fish. Specimen 8 inches (20.3 cm) in length from the Tamar River (from G.P. Whitley, 1941).

Bruce (Pers. comm; 1994) is of the opinion that Cheilodactylid sp. have an extended neustonic larval phase and contends that there is anecdotal evidence that larvae of these species are found at in large numbers up to 250 km off-shore. This researcher suggests that Ladrid sp. may follow a similar life strategy. Successful larval rearing trials conducted as an extension to this study support such a life cylce for *Latris*

lineata. Juvenile *Latris lineata* (Figure 1.4a and 1.4b) display the dark blue dorsal and silver lateral colouration characteristic of many pelagic fish species. Development of this colouration was found to commence at day 26 post hatching (approx. 10mm notochord length) and persisted until these fish died after this study, at approximately age 9 months.

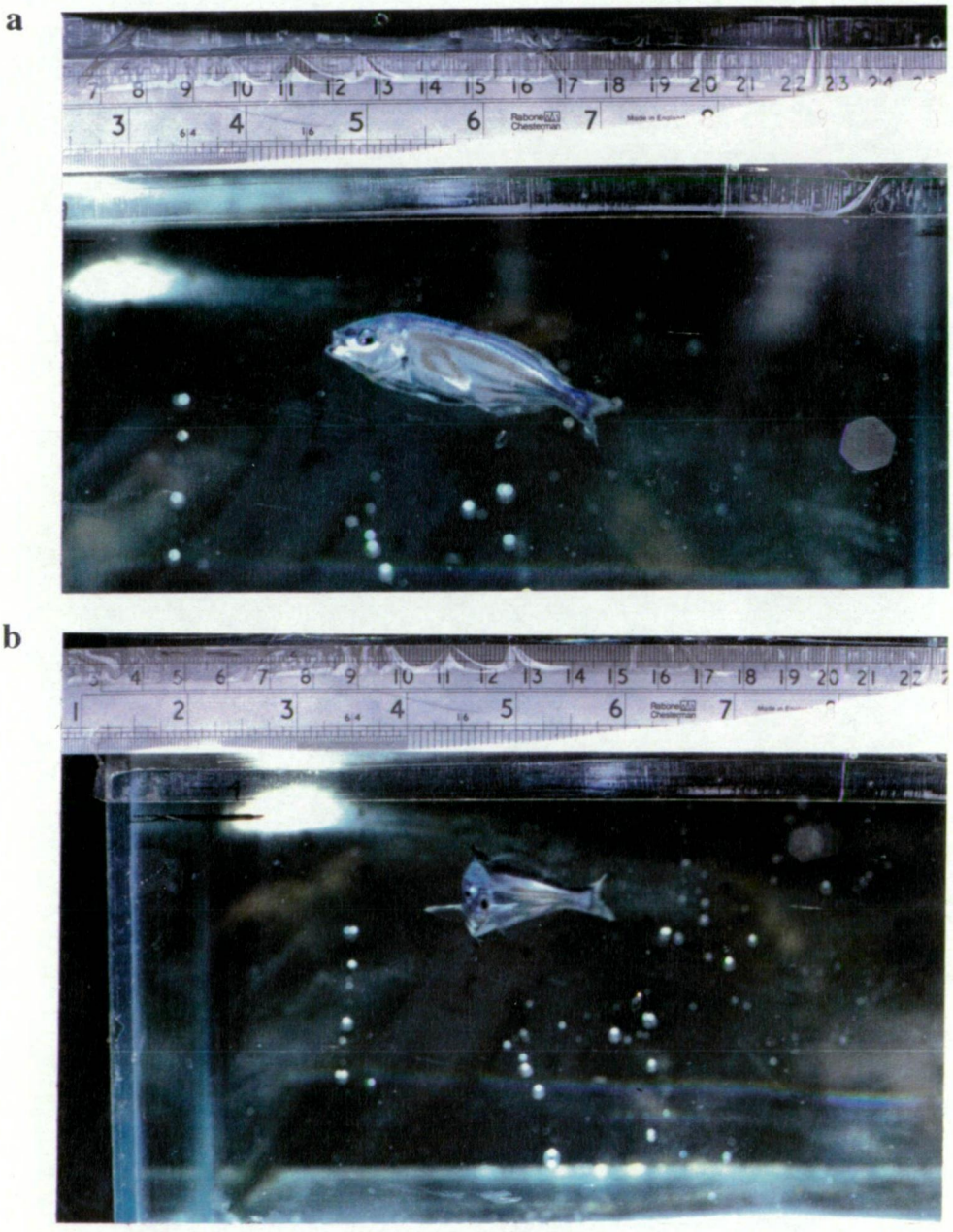


Figure 1.4 Juvenile *Latris lineata* at day 200 post hatching.
(a) Lateral view (b) Anterior view

1.10 Abundance.

Most fisheries information relating to striped trumpeter is dated which is indicative of the progressive decline in the commercial importance of this species in both Tasmania and New Zealand. Johnston (1890) provides a description of the early striped trumpeter fishery in Tasmania including figures showing that 447 dozen (5364) fish of this species were sold at the Hobart fish market during 1888, and 66 dozen (792) fish were exported (almost entirely to Victoria) annually. Fish for the local market were kept alive until sold in perforated fish wells, fitted into the open centre-board whale boats used on the open-sea trumpeter grounds. Trumpeter were known to feed and live healthily in this confinement for a period of up to three months. Similar holding methods are still used in Tasmania for keeping striped trumpeter alive until fishermen return to port, where these fish can be sold live or processed fresh.

Until the 1950's this species was still regarded as being an important commercial species in Tasmanian waters (Roughly, 1951; Parrott, 1959) however examination of fisheries statistics shows that between 1964/5 until 1983/4 the fishery was small with the Australian catch being less than 10 tonnes, except in 1979/80, when 11,354 kg were recorded (Stewart *et al.*, 1991). These figures show that in 1989/90 the Australian catch of striped trumpeter had increased to 78.4 tonnes with 93.4 % (73.2 tonnes) caught in Tasmania and the remainder caught in South Australia. Having spoken to members of the fishing community in South Australia I suspect that the 6.6% (5.2 tonnes) attributed to this state is fact a misnomer, as when South Australians refer to striped trumpeter they are generally refering to the less valued trumpeter perch, *Pelates sexlineatus*, or "shitty".

It is suggested that the decline in commercial importance of this species can be related to a decline in the population of striped trumpeter. Several authors make the point that the number of fish caught has declined as striped trumpeter has been targeted as a

commercial species. In New Zealand, where it was originally a common species, striped trumpeter is now much less abundant (Graham, 1953; Paul, 1986). Although occassionally found in the Bay of Plenty, populations of striped trumpeter have declined except in the southern parts of the south island where striped trumpeter are still common around Stewart Island (Doak, 1972; Ayling and Cox, 1982). A similar situation is apparent in Tasmania as indicated by the absence of the juvenile fish in inshore waters in all but the remote areas of the state (Last *et al.*, 1983). The unspecified "enormous quantities" (Doak, 1972) of striped trumpeter caught in the early years of exploitation are past and this species can best described as a very limited commercial resource.

Doak (1972) wonders "what there is in this fish's biology that makes it particularly susceptible to commercial fishing pressure". Graham (1953) has previously considered the same problem and concluded that it is because striped trumpeter "is such a good biter". This behavioural feature combined with a restricted distribution in which schools confine themselves locally to particular reef systems (as previously described) does not make it difficult to envisage the effect of commercial fishing on such a species. Essentially whenever a new rocky bottom fishing ground is located, striped trumpeter is the first fish to be found and the first to be fished out from that location (Graham, 1953).

1.11 Eating qualities.

Striped trumpeter has long been highly regarded as a food fish. Johnston (1890) states that "the Hobart trumpeter is undoubtedly the king of Tasmanian fishes and is generally esteemed as the finest of Australian edible fishes. Whether fresh, smoked, dried or salted this species could command a ready market in Tasmania or the neighbouring colonies to which it was exported". Smoked striped trumpeter was considered by many "to be equal, if not superior to the Finnon Haddock of Scotland

(*Gadus aglefinnus*)" (Johnston, 1882). In his selection of the six finest food fishes in Australia, Roughly (1951) includes the Tasmanian trumpeter, recommending its firm white flesh, splendid texture and delicious flavour. A similar view is shared in New Zealand (Graham, 1953; Parrott, 1957) with Hector (1872) refering to kohikohi (the Maori name for striped trumpeter) as "the best flavoured of any of our fishes".

2 FURTHER BIOLOGICAL INFORMATION FOR *Latris lineata*.

2.1 Length, weight and condition factor.

The relationship between length and weight for striped trumpeter has not been reported. While commercial fishing operations are in progress, measurement of weight data is difficult due to the constant rolling of the deck even in calm conditions. Time-averaging balances are available (Childress *et al.*, 1980) which enable accurate weight measurement under these conditions, however no such instrument was available for this study. For these reasons, weight measurement was only possible on three occasions (25 May 1990; 12 July 1990 and 4 October 1991) when fish were kept alive in wells and transported to port for processing. Total wet, fish weight (g) was measured (n=65) to the nearest 20 g using a top pan balance (Wedderburn, 12 kg capacity).

The relationships between length and weight for *Latris lineata* shown in figures 2.1 and 2.2. have been compiled from data collected (Appendix 1) on the three sampling occasions described. Data for male and female fish sampled are presented in Figures 2.3, 2.4 and 2.5. These data have limitations in that only a small number of fish was captured on the day preceding each of these sampling dates. Fish sampled on 25 May 1990 (n = 20) and 12 July 1990 (n = 25) were found to be pre-spawning fish, while those sampled on 4 October 1991 (n = 20) were spawning fish. The year interval between these samples must also be regarded as a limitation.

Two way analysis of variance (ANOVA) using Statview 512^{+}(used for all statistical analyses) in which homogeneity of variance was assumed, revealed that there was no significant difference ($P > 0.05$) between mean fork length (cm) of male and female fish (Appendix 2a), nor was there a significant difference ($P>0.05$) between mean lengths of fish sampled on different months (Appendix 2b). Similar ANOVA

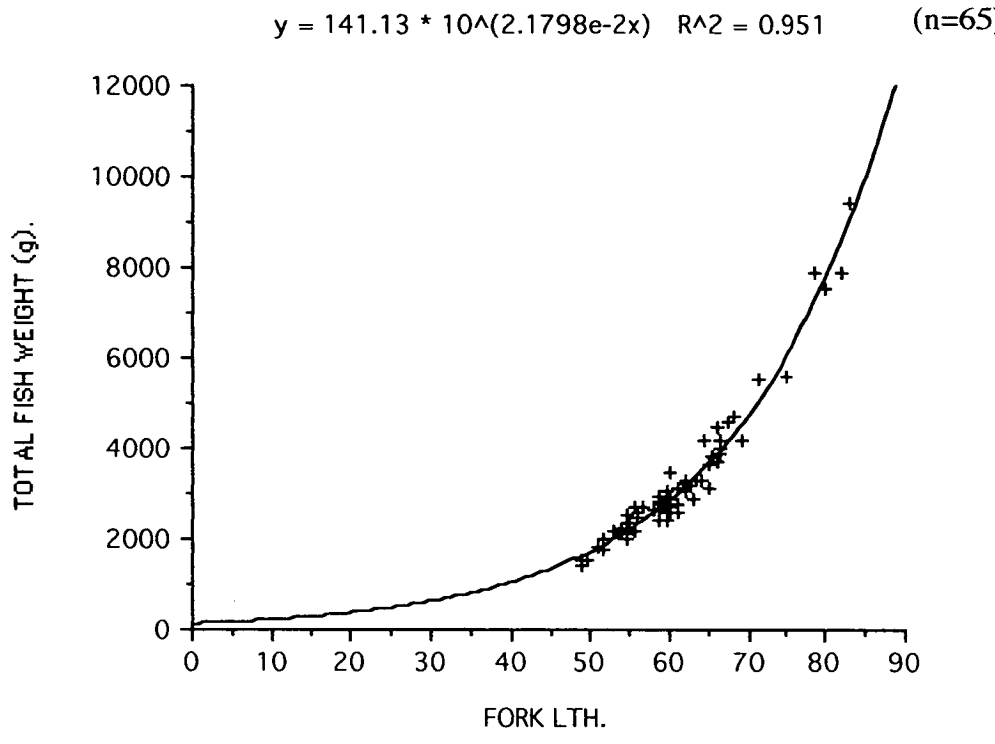


Figure 2.1. Relationship between fork length (cm) and weight (g) for *Latris lineata*. Data compiled from three sampling times (Total n = 65).

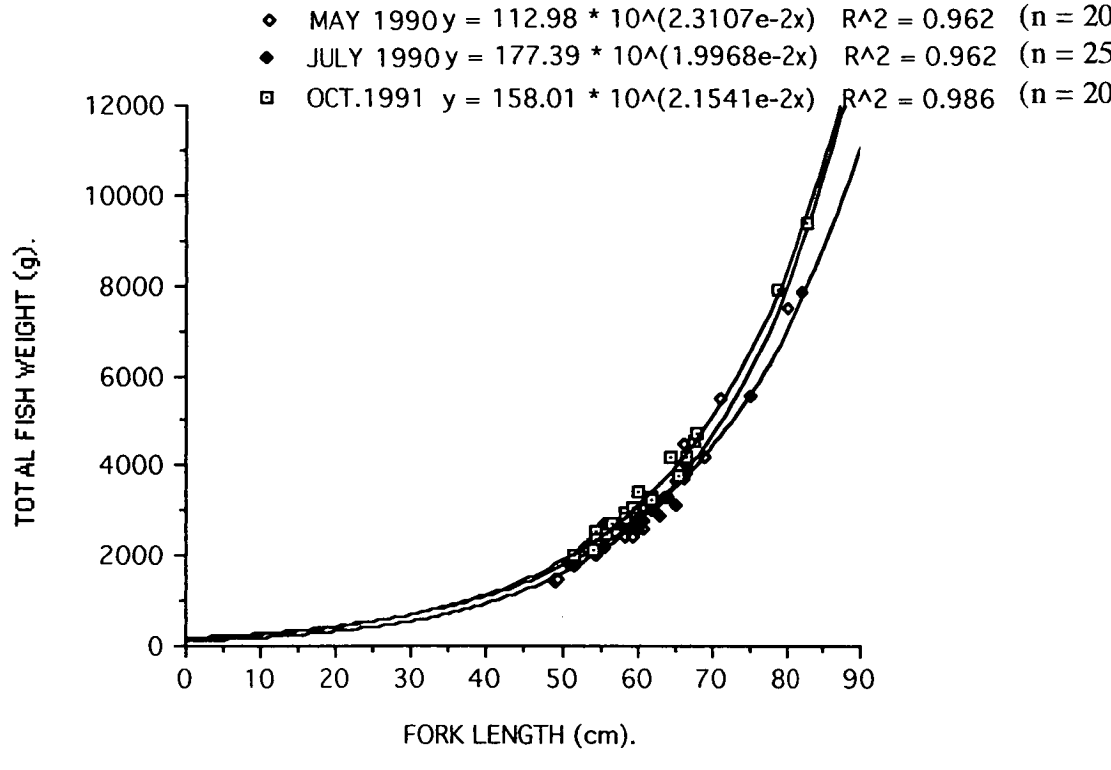


Figure 2.2. Monthly relationships between length (cm) and weight (g) for *Latris lineata*.

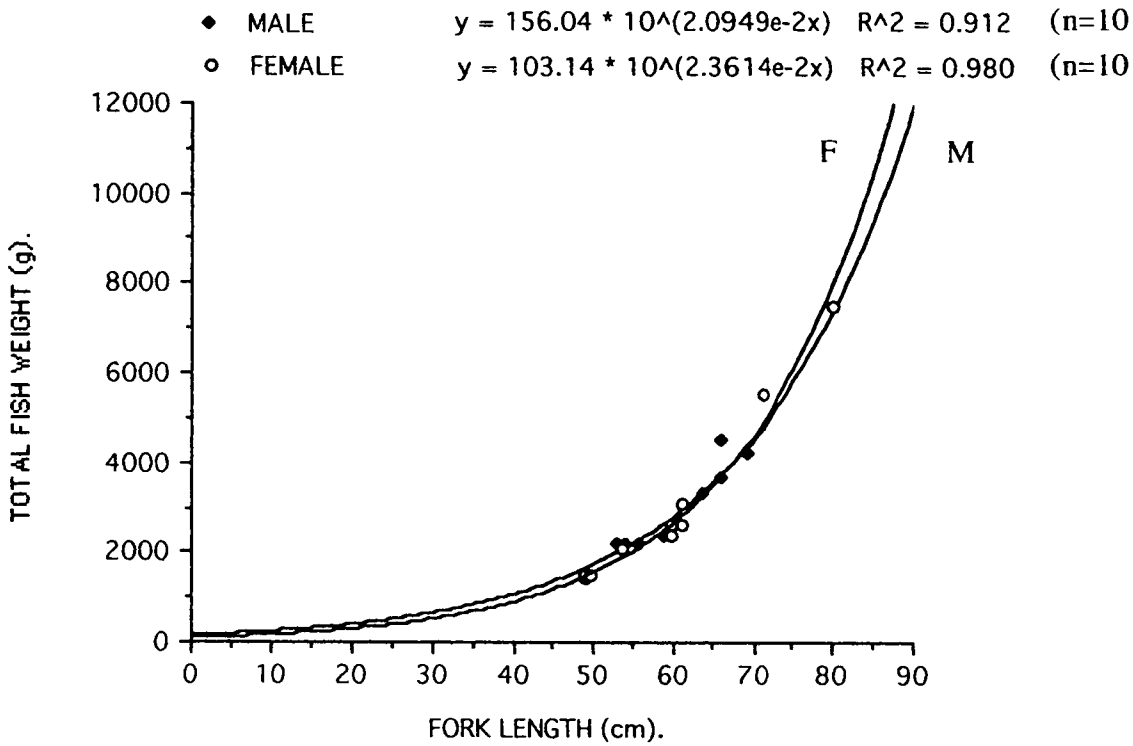


Figure 2.3. Relationship between fork length (cm) and weight (g) of male and female *Latris lineata* sampled on 25 May 1990.

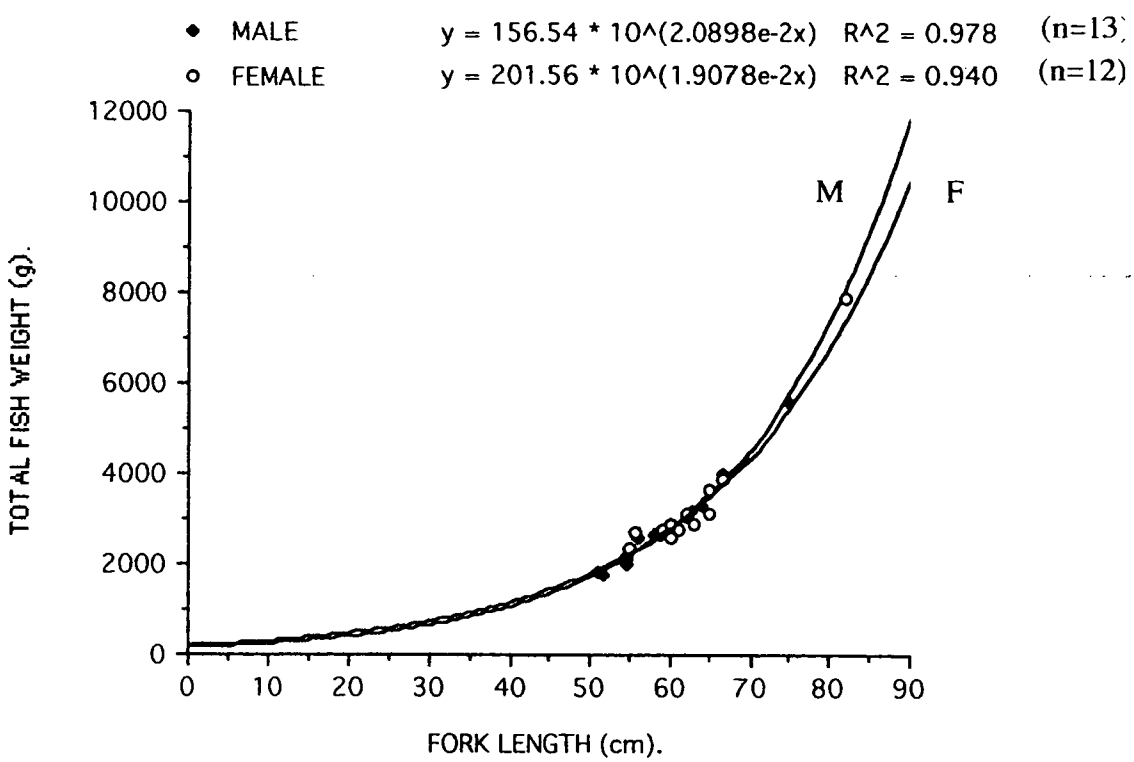


Figure 2.4. Relationship between fork length (cm) and weight (g) of male and female *Latris lineata* sampled on 12 July 1990.

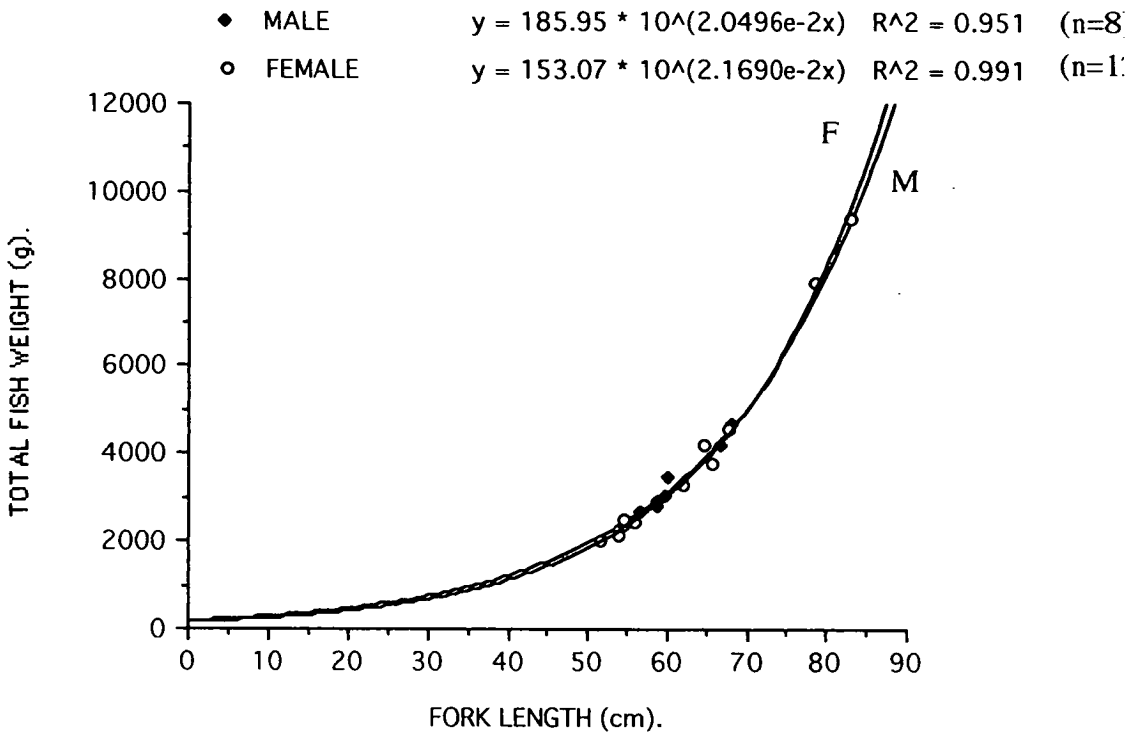


Figure 2.5. Relationship between length and weight of male and female *Latris lineata* sampled on 4 October 1991.

(Appendix 2c) of month, sex and condition factor ((weight (g)/fork length^3(cm) x 100); Priede and Secombes, 1988) using tranformed data (arcsin 1/x) revealed that mean condition factor is significantly different ($P<0.05$) between months (Table 2.1).

Further one way ANOVA comparing sampling months (Appendices 3a, 3b and 3c) shows that mean condition factor of pre-spawning fish sampled on 25 May 1990 and 12 July 1990 are not significantly different ($P > 0.05$). However, both of these sample groups differ from fish sampled during the spawning season (4 October 1991), at which time fish had a significantly ($P < 0.05$) higher mean condition factor.

Table 2.1 Mean condition factor of *Latris lineata* sampled at different months (N =65)

SAMLING MONTH	MEAN CONDITION FACTOR	+/- STANDARD DEVIATION	N
MAY	1.31 [a]	0.125	20
JULY	1.38 [a]	0.065	25
OCTOBER	1.47	0.088	20

Monthly mean condition factors sharing a common super-script are not significantly different (P>0.05)

These data show that the absence of any visible sexual dimorphism in *Latris lineata* extends to the relationship between length and weight recorded for males and females, in the months represented. It is expected that the monthly variations present are due to the over-riding annual patterns which affect the condition of *Latris lineata*, as shown by these data. Although data for pre-spawning and spawning fish are from different years, it is not expected that variations within years would account for this difference in condition factor. A more likely explanation would be that the higher condition factor of fish sampled in October reflects the increase in gonad weight associated with spawning, although no conclusive outcome can be stated given the limitations of these data.

2.2 Size structure of fish sampled.

The fish sampled in this study (N = 334) had a restricted size range between 42.5 cm FL and 85.0 cm FL (Appendix 5). Of these fish 88.6% were further restricted to the size interval between 50.0 cm FL and 69.5 cm FL (Figure 2.6; Appendix 10). The

low representation of small fish may be attributed to sampling selectivity caused by the combined effects of:

a Sampling method.

The size 8.0 hooks used in all capture methods inevitably select for the larger size of fish they are designed to catch.

b Population distribution.

As previously reported in section 1.9, it is generally accepted that juvenile fish inhabit inshore reefs moving out to deeper reefs, of the type fished in this study, as they mature.

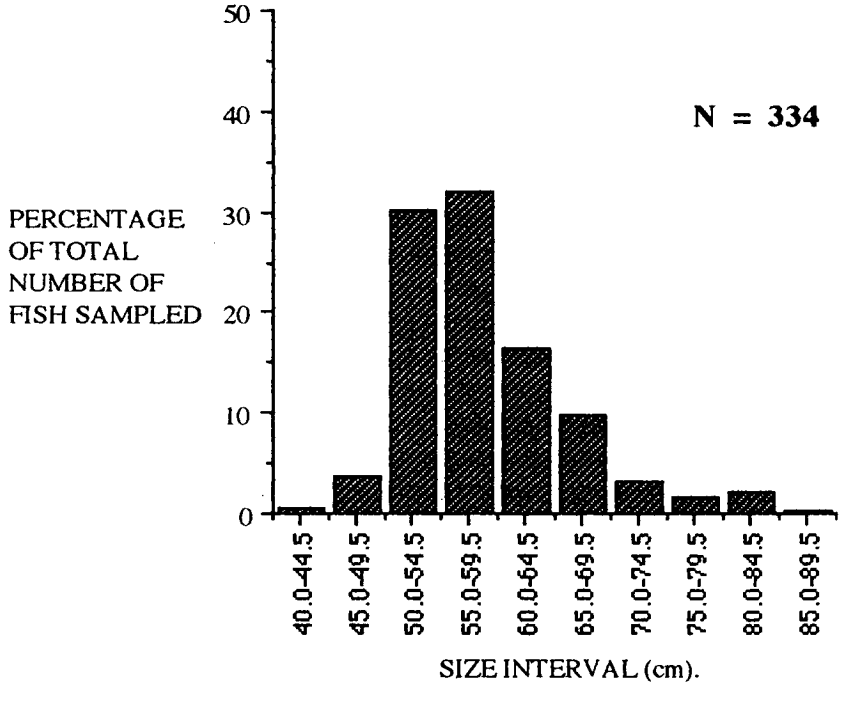


Figure 2.6 Size structure (Fork Length in cm) of all *Latris lineata* sampled in this study.

2.3 Relative gut length and weight.

An indication of the diet of a fish species can be provided by the structure and length of the digestive tract. In general gut length correlates with diet, with herbivores having

longer digestive tracts than carnivorous species. The intestine of carnivorous fishes is shortened reflecting the higher digestibility of the food types consumed (Smith 1982). The digestive configuration of the stomach, mid-gut and hind-gut of striped trumpeter approximates the Y-shaped configuration described by Smith (1989) for Rainbow trout, *Oncorhynchus mykiss*. Four large pyloric caeca are found in this species as indicated in Figure 2.7.

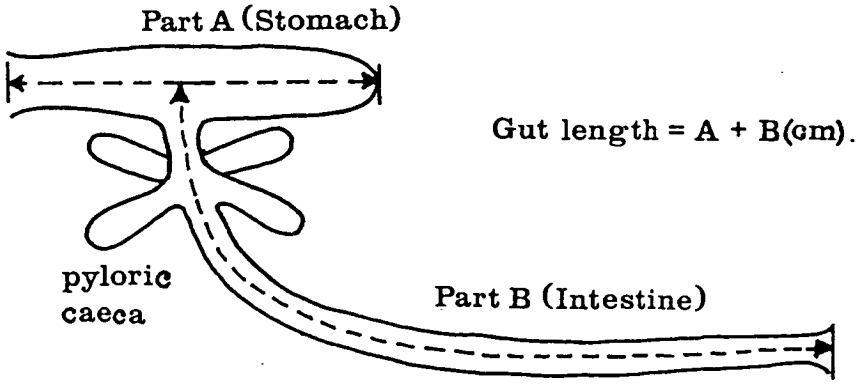


Figure 2.7 Digestive tract of *Latris lineata*, showing measurements for gut length.

To quantify the digestive tract of striped trumpeter, measurements of gut length and total gut weight were recorded from 20 fish sampled on 25 May 1990 (Appendix 1). Gut length was taken as the total available gut length, which was measured as shown in Figure 2.7. Gut weight was made up of all organs removed during processing to present head on, gilled and gutted fish and thus includes the gills and gill arches and the gut contents. From these measurements relative gut length (gut length (cm) / fork length (cm), Smith, 1982) and percentage gut weight (gut weight (g) x 100/ total fish weight (g)) were calculated and are presented in Table 2.2.

Table 2.2 Mean relative gut length and mean % gut weight for *Latris lineata* (N = 20).

	Mean	+/- Standard Deviation
Relative gut length	1.09	0.10
% Gut weight	10.44	1.04

A mean relative gut length of 1.09 compares with carnivorous species reported in data presented by Smith (1982) and agrees with the earlier description of the types of feed consumed by striped trumpeter. The mean percentage gut weight of 10.44 % represents a loss which will be significant for this species if commercial culture is developed and would be expected to be reduced by a period of starvation preceding processing.

2.4 Age of *Latris lineata.*

The determination of age of *Latris lineata* has not been reported. Although not considered a priority in this research project the opportunity existed to collect a small number (N= 50) of otolith samples. Otoliths were chosen for age determination for no reason other than to gain experience in undertaking this form of hard part analysis. Samples were collected from fish processors; Mures in Hobart and Launceston Seafoods in Launceston. Following filleting by processors, fork length of each remnant fish frame was measured and otoliths were removed from the head. Unfortunately capture location and sex was not available for these fish. In a preliminary study a fish which had died in the holding tank was dissected. This dissection revealed the location of the otoliths housed in paired ampullae or otolith chambers, of the labyrinth organ. The ampullae were positioned in depressions within

the cartilage directly beneath the brain as shown in Figure 2.9. Three otoliths were identified in each ampulla, the largest of these otoliths, the sagitta, is contained in the sacculus (Pannella, 1974) and it is this otolith which was collected for age determination.

To remove otoliths from the head two cuts were made at positions A and B in Figure 2.8 with the aid of a hacksaw. This process allowed the removal of a triangular section of the head and exposed the brain. Sagittal otoliths could be located by plunging a pair of fine tipped forceps through the optic lobes of the the mid-brain (Figure 2.9). The hard sagittae could easily be felt against the softer cartilage and were withdrawn. Each pair of sagittal otoliths was cleaned, dried and stored in small paper envelopes on which sample specific data was recorded (Williams and Bedford, 1974; Yabuki,1989; Beckman *et al.*, 1990; Baker and Timmons, 1991).

Temperate fish species are known to show marked seasonal rings within the structure of calcified tissue such as the otoliths (Pannella, 1974). The crystalline material composing the otolith forms an opaque zone during the period of the year when growth of the fish is rapid. At times of little or no growth a translucent (hyaline) zone is deposited. During the early years of development opaque zones are much wider than the hyaline bands while in latter years these opaque bands become progressively narrower (Williams and Bedford, 1974). Interpretation of this annual periodicity allows age to be determined from these patterns deposited during the growth of otoliths. Due to variations in experience of the interpreter, and the growth patterns of populations and individuals, the age of fish as determined from prepared otoliths must not be regarded as being absolute (Pannella, 1974).

Various methods of otolith preparation and treatment have been developed to assist in the age assessment of fish from these calcified structures. Such methods include direct observation of illuminated whole otoliths, breaking and burning otoliths, staining and

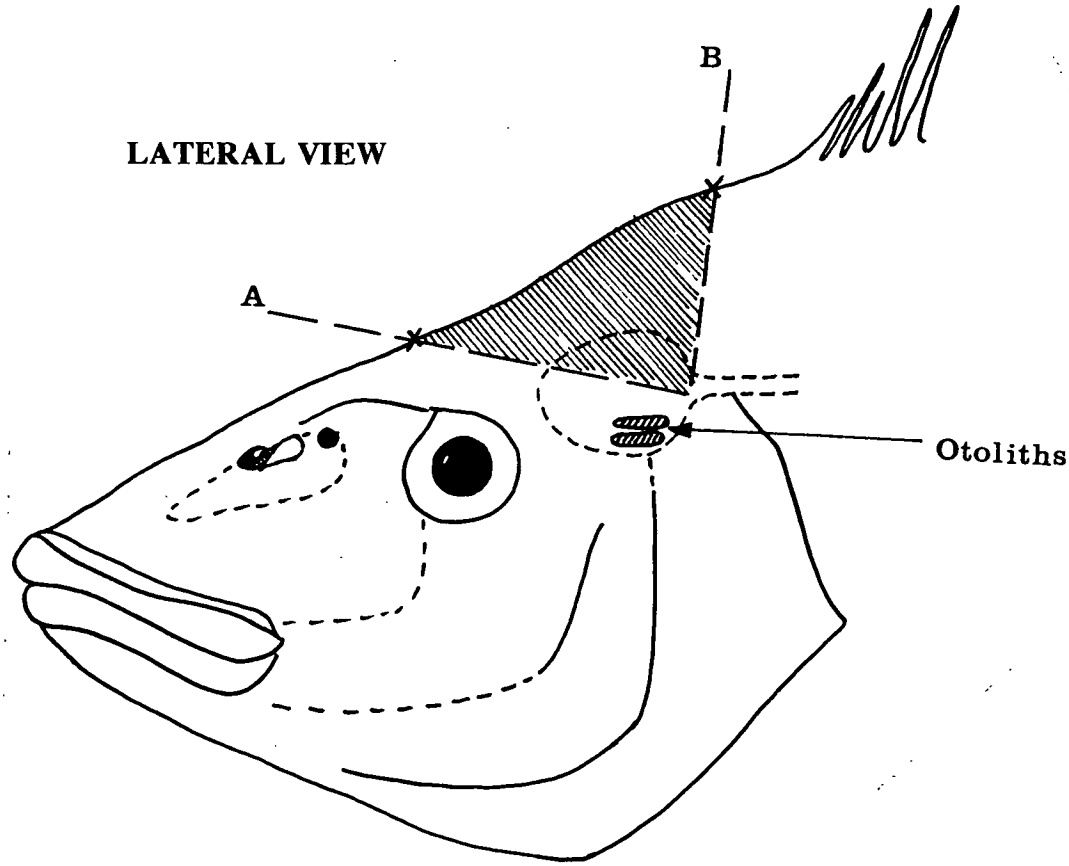


Figure 2.8 Position of cuts (A and B) used to expose the brain of *Latris lineata* for removal of otoliths.

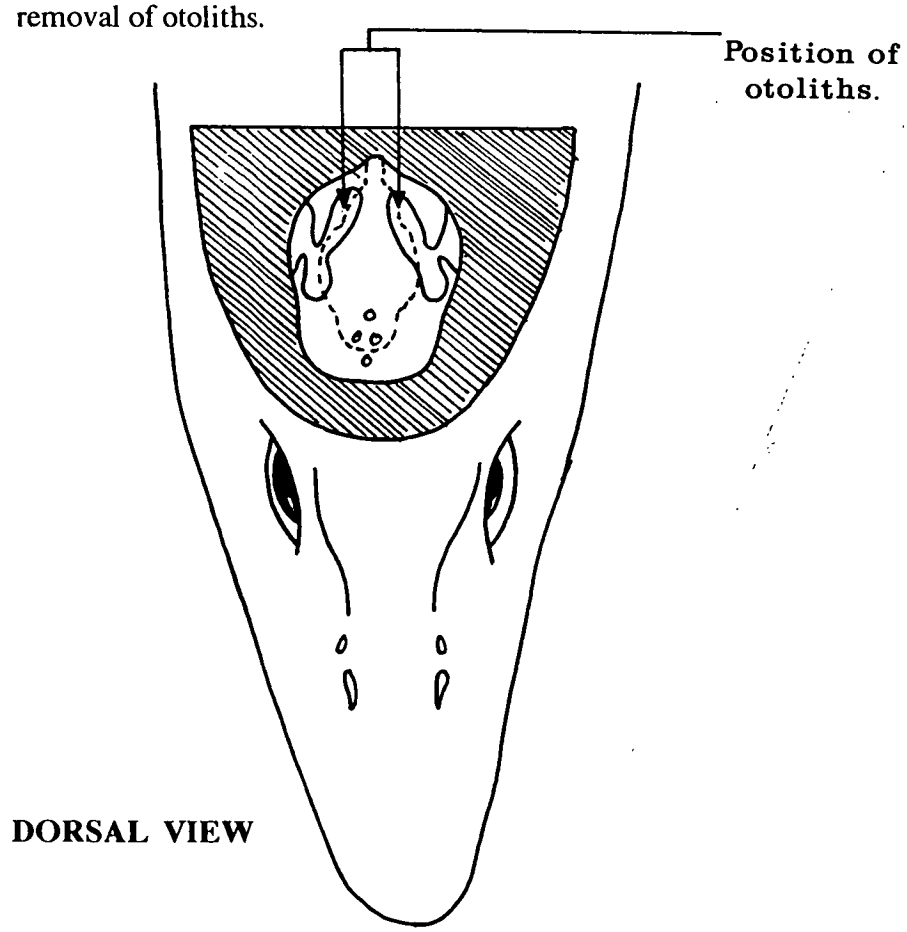


Figure 2.9 Location of otoliths beneath the brain of *Latris lineata*.

sectioning of mounted otoliths (Williams and Bedford, 1974; Casselmann, 1987). Direct observation of whole otoliths using a low power microscope was not successful for *Latris lineata*, as the otoliths are relatively small and dense. It was decided that a method involving the mounting and sectioning of otoliths would provide the most reliable method of interpreting age from these structures.

Processing of sagittae was conducted at the South Australian Research and Development Institute (SARDI) laboratories at West Beach in Adelaide. A polyethylene mould approximately 10 mm deep, 80 mm in length and 50 mm wide was generously sprayed with a silicone-releasing agent Slipicone ^{R} (Dow Corning). Clear polyester resin (Noel Punt S.A. Pty. Ltd; 32% w/w styrene) was initially measured out (20 ml), then mixed with 0.5 ml of catalyst (45% MEKP in dimethyl phthalate). After mixing, the mould was half filled with the polester resin and it was left to set. After two hours of hardening the resin was still slightly "tacky". This allowed one of the sagittae from each sample to be positioned, convex side up, on the resin base. Sagittae were arranged in rows with nuclei of each aligned within each row. This left a suitable distance at one end of each block for secure clamping during sectioning. The arrangement of numbered samples (25/block) was recorded on a diagram of each block. A further 20 mL of resin was prepared and gently poured onto the base to cover the sagittae. The final alignment of nuclei along each row was checked before leaving the resin to set (Figure 2.10).

After 24 hours the resin had completed hardening a top line was drawn across each block with a permanent marking pen and the block was then removed from the mould (Figure 2.11). Another line was then drawn across the top, on the back of each block. These two lines served to define the orientation of samples along each row and later appeared as adjacent marks on the sides of each cut section. Blocks were sectioned using an Accutom-2 (Struers Tech, Denmark) laboratory precision cut-off machine fitted with a 100 mm gem cutting wheel rotating at 500-600 rpm (Figure 2.12). Each

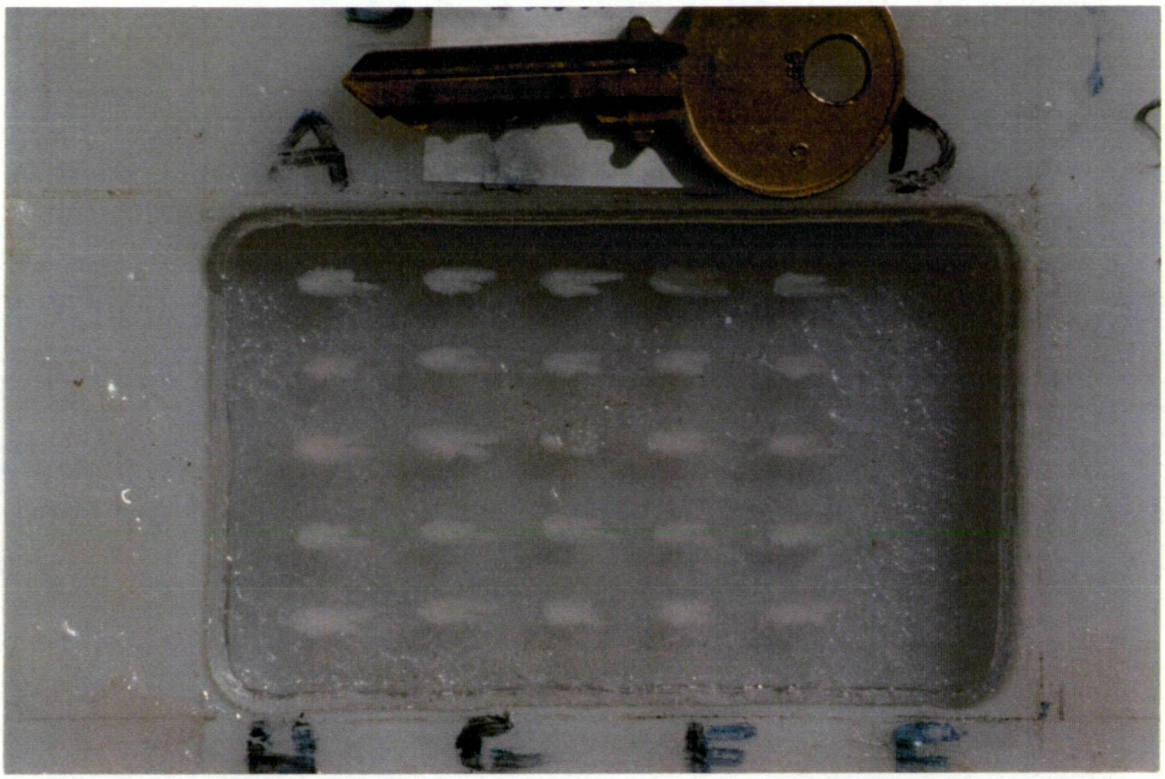


Figure 2.10 Arrangement of otoliths within the clear polyester resin block.

Figure 2.11 Marked polyester resin block with mounted otoliths following the 24 hour setting period and removal from the mould.

row of sagittae was sectioned sequentially through the aligned nuclei to provide 2-3, 0.5 - 1.0 mm thick strips, each of which was labelled and recorded on the corresponding block diagram.

Each strip was ground on a flat surface (bench top) using a wetted sheet of P-400 grade water proof abrasive. Strips were ground on both sides until they were approximately 0.2-0.3 mm thick. Each strip was then ground using a wetted sheet of 3M ^{R} 30 μm grade 261X Imperial lapping film to give a fine finished. Finished strips (Figure 2.13) were wiped with immersion oil (Zeiss 518C) and relabelled prior to storage.

Age assessment from prepared sections requires the subjective interpretation of breaks and changes in the different zones (Casselmann, 1987). To assist interpretation each strip of sectioned sagittae was brushed with immersion oil and inverted to allow it to adhere to a glass slide. Strips were observed with a transmitted light source using a stage microscope at 100 X magnification. Annual zones defined as "annuli", were observed as translucent zones interupting opaque zones. As previously stated the sagittae of striped trumpeter are small and dense. This presents difficulties for the interpretation of annuli, particularly the initial opaque zones representing the early years of growth (1 - 3 years). To overcome this problem sections must be ground to provide thin sections (<0.2 mm) to allow acceptable resolution of these early opaque and translucent zones. All samples were assessed by randomly selecting prepared sections without replacement. This process was repeated until the age of each sample had been assessed at least three times. When discrepancies were found between assessments the most common age was selected.

Assessment of age from sectioned sagittal otoliths of *Latris lineata* provided the data (Appendix 4) presented in Figures 2.14 and 2.15 October 1 was nominated as the birthday of all fish used in this investigation as this approximates the peak spawning

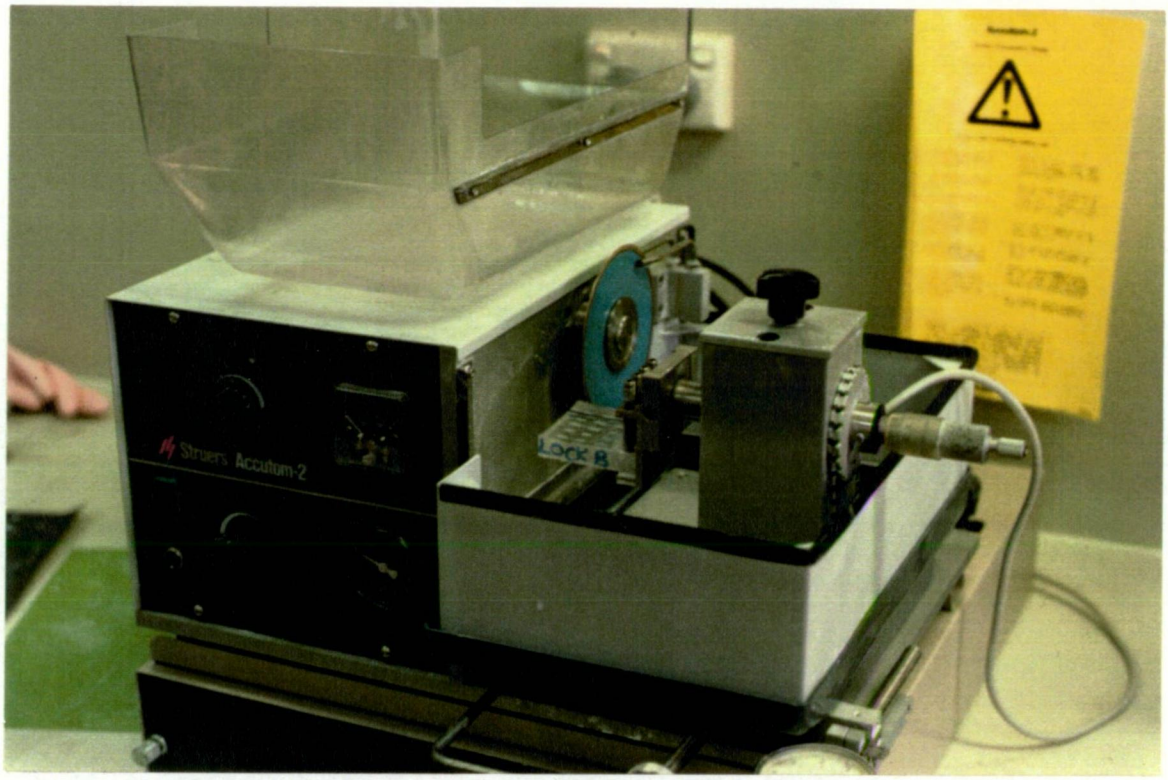


Figure 2.12 Struers Accutom-2 precision cut off machine used to section otolith samples mounted in polyester blocks (see mounted block shown).

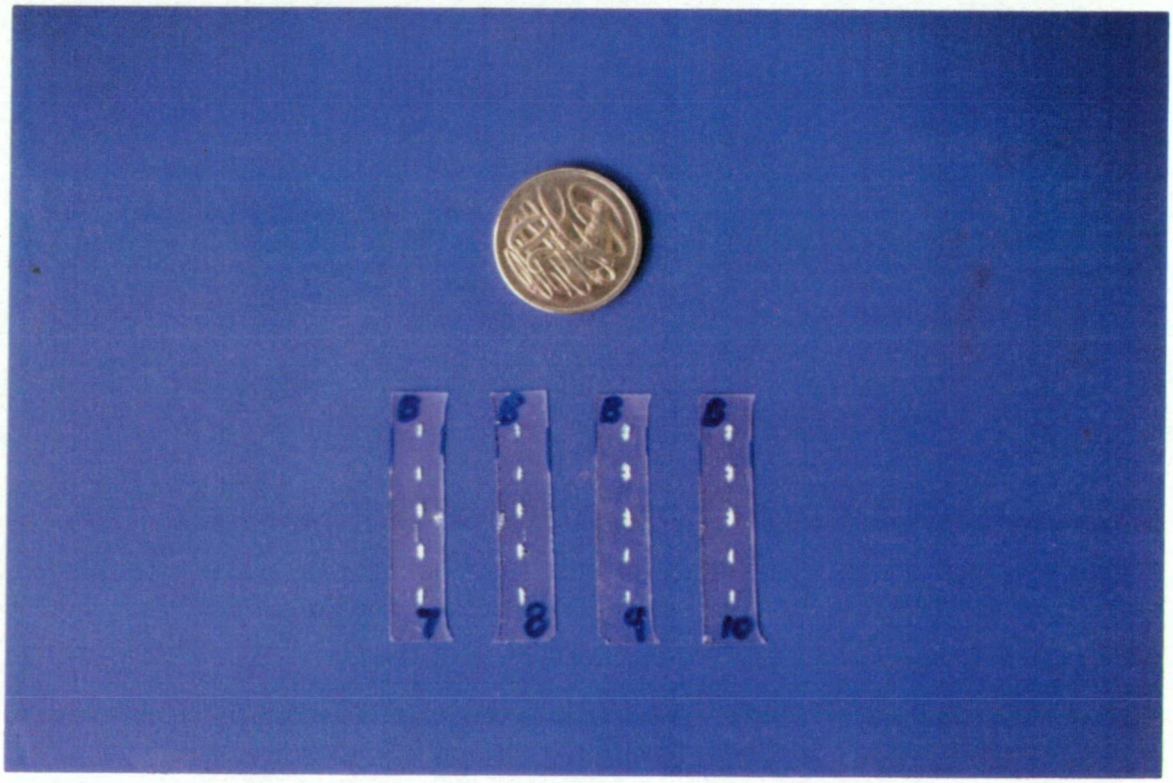


Figure 2.13 Finished otolith sections following cutting and grinding.

period identified for *Latris lineata*, in this study. To the number of annuli recorded, the interval from this birth date, to the date of sampling (expressed as a decimal fraction of a year) was added, to provide the age of each fish sampled. The smallest fish sampled was 43.5 cm FL which recorded an age of 5.7 years, while the largest fish was 78.0 cm F.L. and was determined to have an age of 20.7 years at the time of sampling.

Untransformed data provided the relationship between age and fork length presented in Figure 2.14. A $\log_{10}$ transformation of fork length was used to construct the second order polynomial relationship (Figure 2.15) which provided the best description (R^2=0.916) of the relationship between fork length and age, within the size range represented by these data.

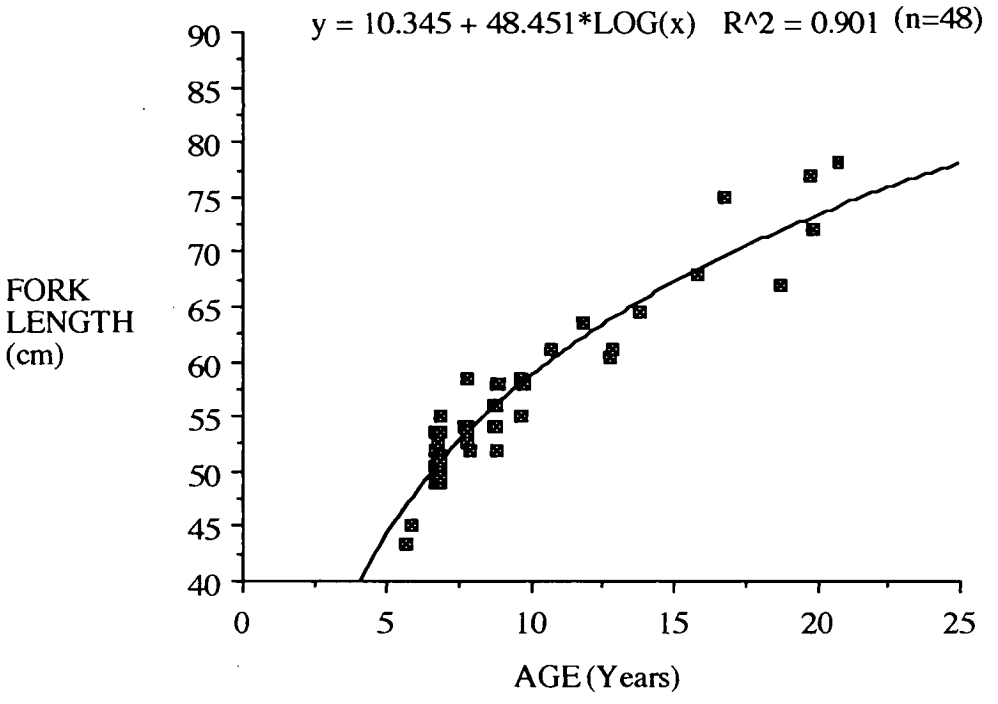


Figure 2.14 Relationship between fork length (cm) and age (years) for *Latris lineata*.

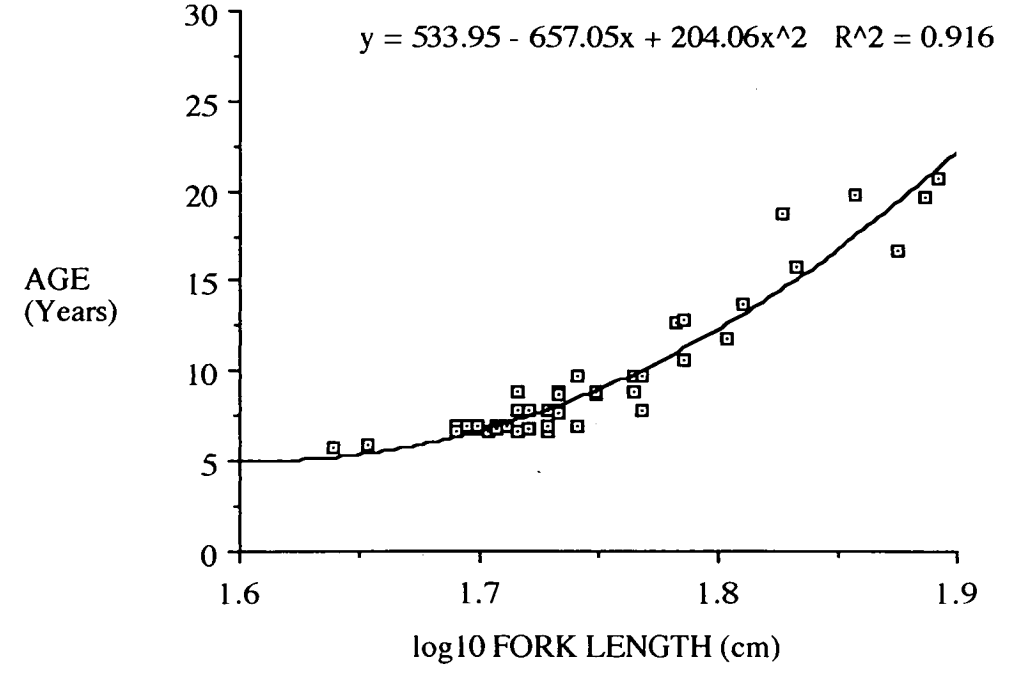


Figure 2.15 Relationship between age (years) and $\log_{10}$ fork length (cm) for *Latris lineata*.

3 REPRODUCTIVE DEVELOPMENT IN TELEOSTS.

A description of reproductive processes in teleosts precedes discussion on reproductive development in *Latris lineata*. Important to this description is an understanding of the ontogeny (formation) of male and female gonads, the structure and function of these organs and the formation of gametes within them.

3.1 Gonad ontogeny.

Teleost gonads arise from single primordia, in the dorsolateral lining of the peritoneal cavity, the primordia is bilateral such that generally one gonad forms on each side (Hoar, 1969). Horvath (1985) describes the position of teleost gonads in the early stages of ontogeny as forming in the angle formed by the swim bladder and the body wall. The sheet-like thickening arising from the peritoneal epithelium infolds with its adnexal connective tissue forming an undeveloped gonad composed of a complex series of ripples or folds (Horvarth, 1985). Unlike other vertebrates, the gonad develops only from a laterally located cortical portion (cortex) of the peritoneal epithelium and there is no contribution from the interrenal (mesonephric) blastema although the development of the nephric system is intimately associated with that of the gonad. This single origin of teleost gonads is suggested as being responsible for the wide-spread occurrence of intersexuality among the teleosts (Hoar, 1969).

The intimacy between the development of the reproductive and nephric systems is evident in the origin of the male gonoducts. Although in mature male teleosts the mesonephros and the gonad are not connected (Figure 3.1), it is assumed that during phylogeny, the mesonephric duct of the urinary system, was the origin of the gonoduct of the reproductive system. In most vertebrates ova are discharged into the peritoneal cavity to be collected by open ovarian funnels and subsequently transfered to the exterior through oviducts communicating with the cloaca. Ovaries of these vertebrates are naked, having no external covering (gymnovarian condition) and the

ovarian funnels and oviducts are derived from mesonephritic ducts as in the male. The oviducts of female teleosts (Figure 3.2a) do not show this association, as the oviducts are continuous with the external covering of the ovary (cystovarian condition). Oviducts develop from a backgrowth of the peritoneal folds which provided the ovarian tunic (*tunica albuginea*). Eggs released from the ovary exit through the oviduct and ultimately pass to the exterior through the urogenital papilla (Hoar, 1969). There are number of exceptions to this final arrangement. Goodrich (1930) states that in some teleosts the oviducts wholly or partially degenerate (Figure 3.2b) This allows ova to pass into the peritoneal cavity then through resultant pores, or funnels, to the exterior. This situation exists in the Salmonidae, Galaxidae, Hyodontidae, Notopteridae, Osteoglossidae and the cyprinid, *Misgurnus* (Hoar, 1969).

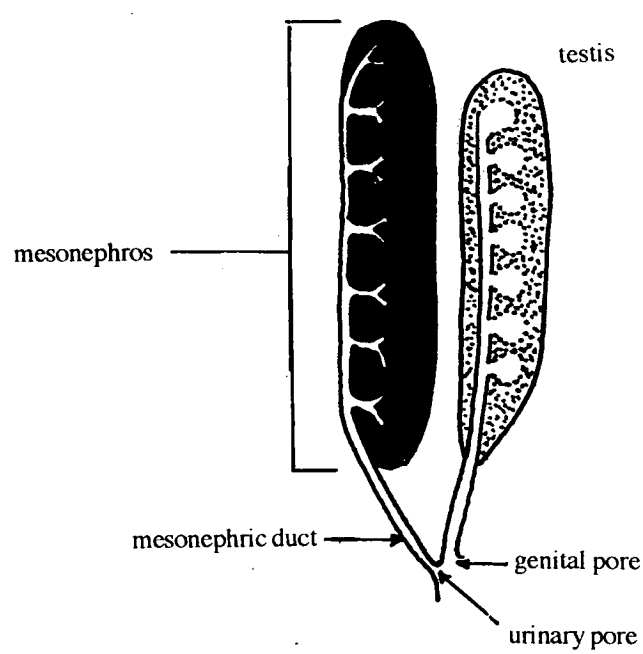
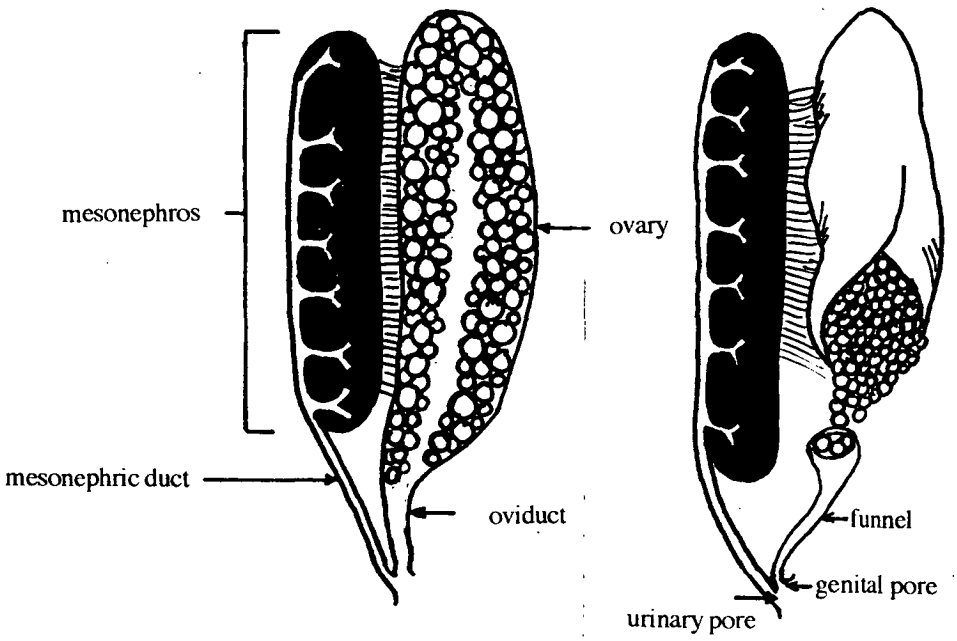


Figure 3.1 Arrangement of the uro-genital system in male teleosts.
(Adapted from Hoar, 1969).



Figures 3.2a and 3.2b Arrangement of cystovarian ovaries of teleosts. (Adapted from Hoar, 1969).

Early in gonad development distinctive primordial germ cells, destined to form gametes, appear conspicuously as larger cells within the proliferating peritoneal epithelium. Primordial germ cells undergo episodes of rapid synchronous mitosis resulting in clusters of identical cells surrounding each germ cell. At this time the gonads have differentiated sexually and differentiation into oogonia and spermatogonia proceeds. Early evidence had suggested a widespread origin of primordial germ cells which then migrated into this modified layer of proliferating mesoepithelium (germinal epithelium) (Hoar, 1969). In all vertebrates investigated the primordial germ cells have been found to have an extragonadal origin subsequently migrating to the genital ridges (Zuckerman and Baker, 1977). Selman and Wallace (1989) summarise more recent findings regarding the origin of primordial germ cells and pre-follicular cells in

teleosts. These authors conclude that the earliest oocytes (oogonia) and the prefollicle cells are derived from the luminal epithelium, as the basal lamina surrounding the germinal ridge is continuous with the basal lamina underlying the luminal epithelium (Figure 3.3). These findings are based on ultrastructural observations of the ovaries from the pipe fish, *Syngnathus scovelli* , in which a sequential pattern of oocyte development is observed to commence at the germinal ridge which contains prefollicle cells, oogonia and oocytes up to the early diplotene stage of meiosis.

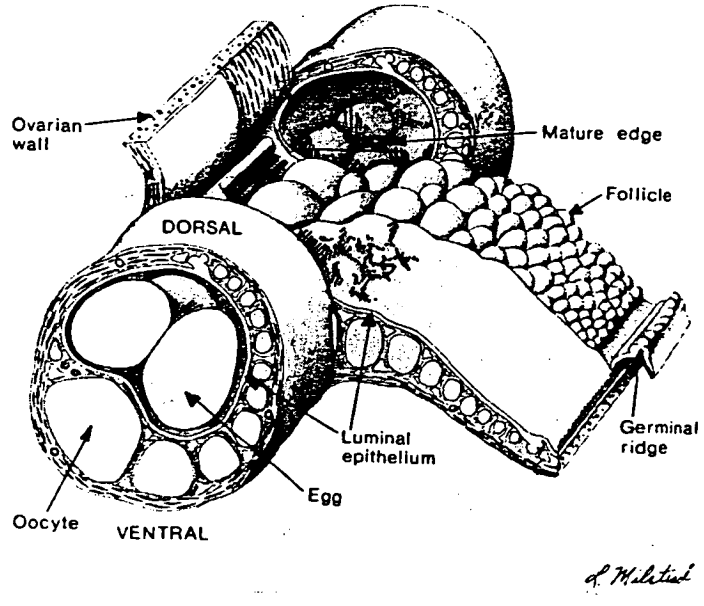


Figure 3.3 Diagram off a cross section of the ovary of the pipefish, *Syngnathus scovelli*, showing unfurled sections illustrating ovarian structure and the association of the luminal epithelium and germinal ridge. (Adapted from Selman and Wallace, 1989).

Ultimately the sex of the developing fish is determined genetically with the differentiation of these gonadal primordium being mediated by gonad steroids. Development to form testes or ovaries is stimulated by androgens or estrogens respectively. Atz (1964) and Yamamoto (1969) agree that as long as primordial germ cells are present, the phenotypic sex of the fish may be influenced by administering exogenous sex hormones. More profound evidence is summarised by Wallace and

Selman (1981) refering to early work of Humphrey (1933) and more recently Blackler (1965) and Blackler and Gecking (1972) in which germ cells from male larvae, transplanted into female hosts, became functional eggs. Conversely germ cells from female larvae, transplanted into male hosts, produced functional sperm. Wallace and Selman (1981) conclude that "the germ cell host simply provides the hormonal and nutrient environment necessary for the development of sperm and eggs ....".

Generally gonadal differentiation occurs earlier in females than males (Nagahama, 1983). Nakamura and Takahashi (1973) found that differentiation of the ovary of *Sarotherodon mossambicus* occurs at about day 20 post-hatch (at rearing temperature 20 °C) while testicular differentiation did not commence until age 50 - 60 days. In salmonids, testicular organisation also occurs latter than organisation in the ovary (Billard *et al.*, 1982). Lebrun (1977) found that in rainbow trout, *Oncorhynchus mykiss*, meiotic oocytes were visible from 40 days post hatching (no water temperature provided) while formation of the seminiferous lobules was not observed by Hurk *et al.* (1979) until 250 days post-fertilization (water temperature 12 °C). Bromage and Cumaranatunga (1988) state that sexual differentiation in rainbow trout *Oncorhynchus mykiss*, occurs at approximately 60 days post fertilization at 10 °C which is soon after hatching. Yoshikawa and Oguri (1978) found that gonadal differentiation in red-belly tilapia, *Tilapia zilli*, occurs in the ovaries at 15 days post-hatching and not until 30 days post-hatching in the testes.

3. 2 Gonad structure.

3.2.1 Structure of the testis.

Testes are usually elongated paired organs which lie in the dorsal part of the body cavity and and which are prolonged posteriorly by a main sperm duct (vas deferens) arising from the posterior mesodorsal surface of each testis. The vas deferentia of each

testis may be followed anteriorly for a variable distance in a connective tissue groove associated with the spermatic blood vessels and nerves. Mature spermatozoa exit through the vas deferens which leads to the urogenital papilla between the rectum and the urinary duct (Hoar, 1969; Billard *et al.*, 1982; Nagahama, 1983).

Although a wide range of structures for male gonads have been developed within the teleosts , two fundamental types of testicular structures, tubular and lobular are recognised, as shown in Figure 3.4 (Billard *et al.* , 1982; Nagahama, 1983). The tubular type, present in antheriniform fish (Hardy heads, garfishes, flying fishes and allies), is displayed by the guppy, *Poecilia reticulata* . In such testes spermatogonia are found organised in cysts only at the blind end of tubules which orientate between the external tunica propria and an internal central cavity. As spermatogonia proceed through spermatogenesis cysts migrate toward the vas efferens (efferent duct) as there is no lumen within testes of this type (Billard *et al.*, 1982; Nagahama, 1983).

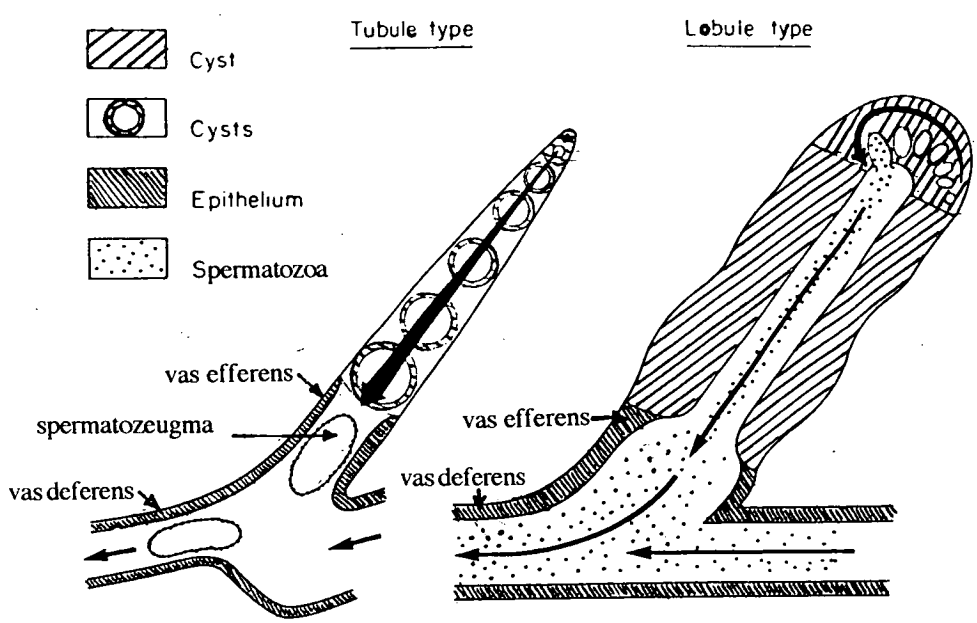


Figure 3.4 Diagramatic structure of tubular and lobular testis. (From Billard *et al.*, 1982).

The more common situation is that of a lobular testicular structure in which nests of spermatogonia proliferate from germ cells scattered along tubules. Each lobule is separated by a thin layer of connective tissue. Spermatogonia within lobules divide mitotically to produce spermatocytes, retained within cysts, which expand and eventually rupture during spermatogenesis. Spermatozoa are released into the lobular lumen which is continuous with the sperm duct (Billard *et al.*, 1982; Nagahama, 1983). At the completion of sperm development the liberated gametes from a multitude of mature cysts are packed within the seminiferous tubules of the testis (Hoar, 1969).

In teleosts the spermatogenic units, lobular or tubular, contain two types of cells bounded by a thin layer of connective tissue, the basement membrane (Figure 3.5) (Hoar, 1969). The first cell type is derived from spermatogonia and gives rise to several generations of spermatogenic cells (Hoar, 1969; Grier, 1981; Billard *et al.* , 1982). The second cell type is somatic and is believed to function as a supporting cell with a nutritive function (Hoar, 1969) as it is supposed that all materials reaching the spermatogenic cells must pass through these somatic cells (Billard *et al.* , 1982).

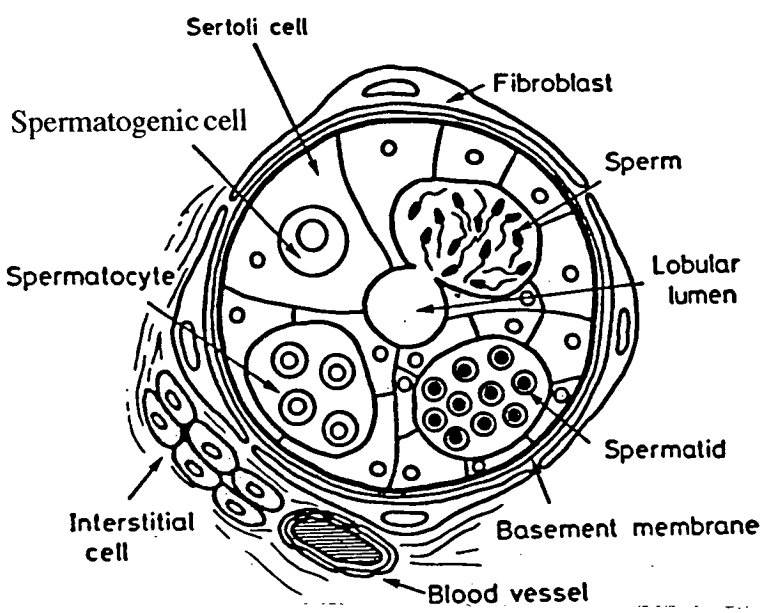


Figure 3.5 Cross section of a lobule showing cell types present. (Adapted from Nagahama, 1983).

Hoar (1969) suggests that the cytology of these somatic cells indicates that they may serve three functions: nutritive, phagocytic (resorption of unused sperm), and production of hormones. Thus they undertake a similar role to the granulosa cells of the ovarian follicle. Grier (1981) also suggests a phagocytic role for these somatic cells which are implicated in the removal of residual bodies cast off by developing spermatids. The nutritive role of these cells is supported by the micropinocytotic vesicles observed along the plasmalemma bordering the basement membrane (Grier, 1981).

Grier (1981), Billard *et al.* (1982) and Nagahama (1983) discuss the confused terminology and functions attributed to these supporting somatic cells. Initially the term lobule boundary cell was adopted (Marshall and Lofts, 1956) in reference to these supposedly Leydig cell homologues from the lobule wall of the Pike, *Esox lucius*. Grier (1981) discards the term "lobule boundary cell" preferring to adopt the name Sertoli cells and suggests a steroidogenic function for these cells in addition to the nutritive and phagocytic roles described. Billard *et al.* (1982) and Nagahama (1983) agree that the term "lobule boundary cell" is not acceptable anatomically and suggest that the steroidogenic functions inferred for these somatic cells was not conclusive enough to regard them as being homologous with the Leydig cells of higher vertebrates. Billard *et al.* (1982) initially adopted the name "cyst cells". They now suggest the alternative name "intralobular somatic cells" as being more appropriate. These authors contend that the somatic cells of the teleost testis contains steroid producing interstitial cells (Leydig cell homologues) present in the interlobular space, together with non-steriod producing interstitial cells. The alternative name "Sertoli cells" is adopted for the somatic cells by Nagahama (1983) on the grounds that it is in wide use with respect to vertebrates and invertebrates, and that this is reasonable justification for continuing to do so until the function of the intralobular somatic cells is established.

In mammals male sex steriods are produced by Leydig cells in the interstitial compartments of the testis. Grier (1981) and Nagahama *et al.* (1982) state that Leydig cells are a typical component of the teleost interstitium together with fibroblasts and the testis blood supply. Grier (1981) suggests that the interstitial cell are "more than likely" to have a steroidogenic function. Nagahama *et al.* (1982) summarise histochemical evidence supporting a steroidogenic function for these interstitial cells, based on the location of the steroid hormone synthesizing enzyme 3β-hydroxy steroid dehydrogenase (3β-HSD), with this enzyme being identified within the interstitial cells from a number of teleost species. In addition, ultrastructural features (mitochondria with typical tubular cristae, agranular endoplasmic reticulum, many lipid droplets) of interstitial cells from a range of teleosts have been observed with the aid of electron microscopy and findings are consistent with ultrastructural features of steroid producing cells. Nagahama *et al.* (1982) conclude that the interstitial cells are homologous with mammalian Leydig cells and are the major sites for the synthesis of male sex steroids.

3.2.2 Structure of the ovaries.

The ovary of teleosts is described by Hoar (1969) as being "a complex hollow organ" and together with those of the basking shark, *Cetorrhinus maximus*, are unique in structure within the vertebrates. The outer most cell layer of the developing ovary forms a visceral peritoneal covering, internal to which develops the fibrous *tunica albuginea* (ovarian wall) (Gupta, 1975). The numerous oogonia arise from primordial sex cells in, or close to, the germinal epithelium which lines both the surface of the ovary and the internal cavity within the hollow teleost ovary (Hoar, 1969). Morrison (1990) observing the ovarian wall of *Gadus morhua* , states that both the inner surface and ovigerous folds are lined with squamous epithelium. According to Brummett *et al.* (1982) this squamous epithelium may not be true epithelium but rather elements of the stroma. Some germinal cells can be seen just beneath this layer

of cells and it is suggested that the stromal elements have moved out to line the inner surface of the ovarian wall and ovigerous folds.

Internally the structure of the ovary is arranged as a series of densely packed ovigerous folds, which may occupy the entire inner cavity. The ovigerous folds are maintained by a highly vascular connective tissue stroma, extending from the well vascularised inner layer of the *tunica albuginea* (Hoar, 1969; Morrison, 1990). These connective tissue lamellae form the substance of the gonad supporting the germinal epithelium cells and primordial germ cells. It is the vascularisation of this tissue which provides the route for the considerable amounts of nutrients required during oogenesis (Beach, 1959). The *tunica albuginea* in *Gadus morhua* is contractile and consists of a layer of collagen, then well-innerviated smooth muscle cells separated by collagen, underneath which is a well vascularised layer (Morrison, 1990).

Fish eggs are released from mature follicles either into the central ovarian lumen or the peritoneum depending on the species of teleost (Hoar, 1969). The stroma of the ovaries of teleosts is rich in elastic tissue and smooth muscle (Hoar, 1969; Morrison, 1990) so that in ripe fish cranio-caudal waves of muscular contraction, assisted by intense contractions of the ventral abdominal wall force the eggs from the body (Brawn, 1961; Harder, 1975).

3.3 Sperm formation.

Within a testis spermatozoa are formed from spermatogonia through the process of spermatogenesis. Unlike mammals, teleosts lack a permanent germinal epithelium. This necessitates that resting spermatogonia form a reserve from which spermatozoa develop during each spawning season (Hoar, 1969; Morrison, 1990). During the process of spermatogenesis, spermatogonia first proliferate by repeated mitotic divisions and growth, resulting in the formation of primary spermatocytes. Secondary

spermatocytes are formed following reduction (meiotic) division and these subsequently divide to form spermatids. Functional sperm or spermatozoa develop following metamorphosis of spermatids in a process known as spermiogenesis (Hoar 1969). Development of germ cells takes place within cysts formed by Sertoli cells in both lobular and tubular testis types (Grier, 1981). Within the cysts at least two types of spermatogonia (types A and B) are located in the basal region. These are separated from the basement membrane by thin, intervening strands of Sertoli cell cytoplasm (Nagahama, 1983). Morrison (1990) studying *Gadus morhua* , reports that small bridges between adjacent spermatogonia are sometimes seen, an observation supported by Nagahama (1983). Each primary spermatocyte divides meiotically into two smaller haploid secondary spermatocytes. Secondary spermatocytes are rarely seen in histological sections as they are quickly transformed, through the second meiotic division, into smaller spermatids (Nagahama, 1983).

Spermatids are transformed into spermatozoa through the process of spermiogenesis in which the nucleus and cytoplasm are reorganised and a flagellum is formed. In some teleosts with tubular testes, at or near the completion of spermiogenesis, the spermatids show an association with the Sertoli cells. However no such association is found in species with lobular type testes (Nagahama, 1983).

After spermiogenesis mature spermatozoa are made ready for discharge (Hoar, 1969). This involves a hormone-dependant thinning, or hydration of the semen, which is referred to as spermiation (Billard *et al.* , 1982). During this process there is a decrease in sperm concentration due to hydration which, it is suggested, increases intralobular pressure allowing spermatozoa to move towards the vas deferens where they accumulate. It is logical to assume the involvement of nervous or neuro-hormonal mechanisms in the release of sperm through the genital papilla, because the short activity time of sperm in water requires that there is a high degree of synchrony between the shedding of gametes by males and females (Billard *et al.* , 1982).

3.4 Oocyte development in teleosts.

The formation and development of oocytes in teleosts is a dynamic process. Unlike spermatocytes, oocytes subsequently enter a period of growth caused mainly by an accumulation of yolk (Nagahama, 1983). Wallace and Selman (1981) and Selman and Wallace (1989) review oocyte growth as it occurs in teleosts, summarising the significant amount of information which has been gathered within this topic. The terminology adopted by these authors is based on that of Yamamoto (1956) and it is this terminology that will be followed in the present review. The development and functions of the oocyte follicle will be considered in section 3.6.

3.4.1 Formation of primary oocytes.

Like spermatogonia, the stem cell population of oogonia proliferate by mitotic divisions and give rise to secondary oogonia. Oogenesis is the term used correctly to describe the transformation of these secondary oogonia into primary oocytes (Wallace and Selman, 1981; Bromage and Cumaranatunga, 1988; Morrison, 1990). As previously discussed in section 3.1, oogenesis occurs within the germinal regions of the ovarian luminal epithelium (Selman and Wallace, 1989). Following this process each oocyte and its associated prefollicle cells, separate from the germinal region to form a primordial follicle (Wallace *et al*., 1987). It is generally accepted that at this time meiosis commences and chromosomes become arrested at late diplotene of the first meiotic prophase (De Vlaming, 1983; Nagahama, 1983; Bromage and Cumaranatunga, 1988; Selman and Wallace, 1989).

3.4.2 Chromatin nucleolus stage.

The chromatin nucleolus stage is characterised by a prominent nucleolus connected with chromatin threads (Guraya, 1986). It is during this stage that DNA replication in presynaptic oogonia occurs (pre-leptotene) and ultimately the chromosomes form synaptonemal complexes (pachytene). Postsynaptic chromosomes finally assume a

"lampbrush" configuration (diplotene) which persists until immediately prior to germinal vesicle breakdown (GVBD) concomitant with final oocyte maturation (Wallace and Selman, 1981; Guraya,1986; Selman and Wallace, 1989). After summarising the work of a number of researchers Guraya (1986) concludes that lampbrush chromosomes form important sites for the synthesis of RNA and protein. Soon after formation, the primary oocyte (chromatin nucleolar stage) contains a single large, centrally located nucleus surrounded by a thin layer of cytoplasm. Externally it is enclosed by the initial follicle, which consists of a few squamous follicle cells (Wallace and Selman, 1981; de Vlaming, 1983; West, 1990).

3.4.3 Perinucleolus stage.

In the early perinucleolus stage the nucleus (germinal vesicle) increases in size and multiple nucleoli form in the peripheral margin. This reflects an amplification of ribosomal genes soon after arrest in prophase (Selman and Wallace, 1989). Lampbrush chromosomes become more apparent at this stage. This suggests prominent transcription of heterogenous RNA destined to form messenger RNA (m-RNA) as it migrates into the oocyte cytoplasm (ooplasm). Guraya (1986) concluded that the multiple nucleoli are the sites of synthesis for ribosomal RNA during oogenesis. Furthermore, this synthesis results in the large number of ribosomes in the ooplasm prior to yolk deposition which are stored for use during future embryonic development.

Characteristic of the perinucleolar stage of oocyte growth in teleosts is the accumulation in the perinuclear cytoplasm of electron dense, basophilic material (Wallace and Selman, 1981; Guraya, 1986). These bodies are variously described as "Balbiani's vitelline bodies" (Guraya, 1986) "yolk nuclei" and "balbiani bodies" (de Vlaming, 1983; Selman and Wallace, 1989), the term adopted in this study. Balbiani bodies first develop in a juxtanuclear position and subsequently leave the nucleus through pores in the nuclear envelope, ultimately they migrate to the periphery of the

ooplasm where their components are dispersed resulting in their disappearance prior to yolk deposition (Wallace and Selman, 1981; Guraya, 1986; Bromage and Cumaranatunga, 1988). Structurally Balbiani bodies in teleosts are a diverse complex of cytoplasmic organelles and inclusions which vary in organisation and development between fish species. Organelles implicated with Balbiani bodies include a yolk nucleus (consisting of RNA and protein), mitochondria, golgi bodies, cisternae of smooth endoplasmic reticulum, lipid inclusions, annulate lamellae and multivesicular bodies. It is believed that Balbiani bodies function as organisational centres for the formation of the cytoplasmic organelles and materials required by the oocyte during previtellogenesis (Guraya, 1986; Selman and Wallace, 1989).

During the primary growth stages (chromatin nucleolar and perinucleolar stages) the oocytes of most teleosts increase in volume approximately a thousand fold. This is observable microscopically as an increase in diameter from 10 - 20 μm up to diameters generally between 100 - 200 μm. (Selman and Wallace, 1989). This represents only a small proportion of the total growth to be achieved during oocyte development. This increase is due to the accumulation within the ooplasm of the various cytoplasmic organelles and RNA species described (Guraya, 1986; Selman and Wallace, 1989). By the end of primary growth the oocyte is enclosed by a differentiated multi-layered follicle. Oocytes of many teleosts show an intimate association with the follicle at this stage, with the oocyte surface extended into numerous microvilli projecting toward the surrounding follicle cells. Precursor material of the vitelline envelope is also seen to commence accumulation during primary growth of teleost oocytes (Wallace and Selman, 1981; Selman and Wallace, 1989).

3.4.4 Cortical alveoli formation.

Commencement of the secondary stages in oocyte growth is marked by the appearance in the ooplasm of vesicles referred to as cortical alveoli. The cortical alveoli are the first distinct structures (i.e. observeable using light microscopy) to form within the

ooplasm of the developing oocyte. Bromage and Cumaranatunga (1988) describe vesicles which initially appear in the periphery and eventually fill the whole cytoplasm in rainbow trout, *Oncorhynchus mykiss*. As hepatically derived yolk is incorporated, these vesicles are displaced towards the periphery and it is suggested that cortical alveoli form from these vesicles in this position. Some authors adopt a stage, refered to as the "circumnuclear ring " stage, which precedes the formation of cortical alveoli. Kjesbu and Kryvi (1989) state that in *Gadus morhua*, the circumnuclear ring is displaced towards the periphery and breaks down as cortical alveoli appear in the cytoplasm. Morrison (1990), also working on *Gadus morhua*, describes electron dense material associated with mitochondria in the peripheral cytoplasm before cortical alveoli are seen. Dense cored vesicles and material similar to that found in cortical alveoli is found closely associated with Golgi bodies during formation of cortical alveoli. It would appear from these reports that cortical alveoli are formed from a precursor structure identifiable by these authors.

Early investigators suggested that the cortical alveoli contained yolk as reflected in the names attributed to them i.e. yolk vesicles, endogenous yolk, primary yolk globules, lipid vesicles or granules, intravesicular yolk, intravacuolar yolk, carbohydrate yolk and vacuolar yolk (Wallace and Selman, 1981; De Vlaming, 1983; Guraya, 1986; Bromage and Cumaranatunga, 1988; Selman and Wallace, 1989). Selman and Wallace (1989) state that as these vesicles do not contain yolk any reference to yolk should not be associated with them. Bromage and Cumaranatunga (1988) share this opinion indicating that a lack of evidence linking these vesicles to the incorporation of true yolk should preclude them from being refered to as yolk vesicles. West (1990) reverts to using yolk vesicles to describe the precursory structures which give rise to cortical alveoli, as described previously. De Vlaming (1983) shares this description, suggesting that yolk vesicles most probably give rise to cortical alveoli.

Recently the use of electron microscopy, histochemical, biochemical and autoradiographic studies have presented a more precise interpretation of the morphology and function of cortical alveoli. From these studies cortical alveoli have been found to accumulate Alcian blue and periodic acid-Schiff (PAS) positive substances (i.e. carbohydrates) during their development. It is suggested that they probably contain mucopolysaccharides (Guraya, 1986; Bromage and Cumaranatunga,1988; Morrison, 1990). Laale (1980) describes the contents of cortical alveoli as being lipid-coated mucoproteins. Studies summarised by Guraya (1986) implicate endogenously synthesized glycoprotein, as being another cortical alveolar component in a number of teleost species. These protein-bound carbohydrate moieties associated with cortical vesicles have recently been characterised as sialoglycoproteins. Mommsen and Walsh (1988), refering to work conducted by Inoue and Iwasaki (1980a), conclude that in eggs of Herring, three main sialoglycoproteins are found. Each of these molecules has the same unique protein backbone, representing 40 -50% of the molecule while the carbohydrate composition is quite variable.

This information supports the generally accepted understanding that cytoplasmic endoplasmic reticulum and golgi bodies are responsible for the formation of cortical alveoli. It is suggested that protein components found in the cortical alveoli are formed in association with the endoplasmic reticulum then transfered to golgi bodies. The golgi bodies are responsible for the formation of the carbohydrates and it is at the golgi bodies that these carbohydrate-protein complexes are constructed (Guraya, 1986). More recently cortical alveoli have also been shown to contain endogenous lectins associated with the vesicle membrane. It is suggested that these lectins function as specific membrane receptors based on the specific sugar binding properties attributed to lectins (Guraya, 1986; Selman and Wallace, 1989).

In latter stages of oocyte development cortical alveoli are displaced by the accumulation of true yolk and lipids within the ooplasm (Bromage and Cumaranatunga, 1988; Selman and Wallace, 1989; Morrison, 1990). At fertilization the cortical alveoli release their contents between the chorion (zona pellucida) and the protoplasmic surface (De Vlaming, 1983; Bromage and Cumaranatunga,1988; Selman and Wallace, 1989). This cortical reaction at fertilization thus leads to the formation of the perivitelline space which widens rapidly to stop possible polyspermy (Guraya, 1986).

3.4.5 Vitellogenesis.

During the secondary growth of oocytes the accumulation of yolk is primarily responsible for the large increase in volume (Wallace and Selman, 1981; De Vlaming, 1983; Bromage and Cumaranatunga, 1988; Selman and Wallace, 1989). Guraya (1986) quantifies this accumulation of nutrient reserves, stating that there is an increase in the weight of the ovaries of many fishes from approximately 1% of body weight compared to 20% or more of body weight being attributed to the ovaries of pre-spawning, "ripe" fish. Further quantification of this accumulation is also provided by Selman and Wallace (1989) indicating that yolk proteins account for > 80-90% of the dry weight of eggs in teleosts. Guraya (1986) identifies three types of yolk in the eggs of fish. Lipid yolk droplets (lipid droplets) appear in oocytes prior to the accumulation of proteid yolk globules (yolk globules). A non-granular fluid yolk is also identified.

3.4.5.1 Lipid droplets.

In some, mostly marine teleosts, lipid droplets begin to accumulate in the cytoplasm at about the same time as the formation of cortical alveoli precursors (De Vlaming, 1983; West, 1990). Lipid droplets are identified as highly sudanophilic inclusions consisting mainly of neutral fats (triglycerides) however the way in which they are formed is as yet unknown. These lipid droplets appear empty in samples prepared following

conventional staining procedures as their contents dissolve when preserved ovarian tissue samples are dehydrated using alcohol (West, 1990). Deposition of lipid droplets occurs prior to that of proteid yolk and is considered to indicate the start of endogenous vitellogenesis (Guraya,1986; Gillis *et al.*, 1990).

Neutral lipids (triglycerides) are found within the cytoplasm and yolk and it is suggested that the lipid yolk is synthesized within the ooplasm with minimal involvement of cytoplasmic organelles, apart from the Golgi bodies or dictysomes. These lipid droplets first form in the perinuclear cytoplasm and then extend outward within the developing oocyte. Initially small lipid granules are formed consisting of triglycerides and phospholipids, as the oocyte grows the phosholipid component disappears (Guraya, 1986). Towards the end of oocyte development and maturation these small lipid droplets generally coalesce to form several larger droplets which are involved in the formation of the oil droplet found in fully developed eggs (de Vlaming, 1983; West, 1990).

3.4.5.2 Endogenous vitellogenesis.

Guraya (1986) presented results of a number of studies and concluded that there is evidence to support some endogenous contribution to the formation of proteid yolk (yolk globules) during early vitellogenesis. This evidence is in the form of observed associations between yolk globule precursors (yolk granules), mitochondria and vesicles produced from Golgi bodies and endoplasmic reticulum. The author suggests that the actual synthetic participation of these organelles has not been shown conclusively and their role may be simply as a site for the condensation of yolk material. Alternatively the ultrastructural descriptions on which these conclusions are based may in fact be describing the formation of cortical alveoli as the exogenous origin of yolk globule constituents is well established.

3.4.5.3 Exogenous, "true" vitellogenesis.

Many teleosts display early life strategies in which developing larvae endure periods of starvation prior to exogenous feeding during which time embryonic and larval survival is dependant on the availability of maternal yolk deposited during oocyte development. The extraovarian provisioning of components of the oocyte, especially yolk, is termed exogenous vitellogenesis (Mommsen and Walsh, 1988). The appearance of proteid yolk in yolk globules is characteristic of vitellogenic oocytes, the yolk globules first occur in the cortical regions then proceeding to fill the ooplasm (Guraya, 1986; West, 1990). Depending on the species of teleost, yolk globules may maintain their integrity during oocyte growth; fuse soon after formation; or fuse at latter stages of oocyte development. In teleosts with pelagic eggs fusion occurs at maturation. This results in the displacement of the ooplasm into a rim surrounding a continuous body of fluid yolk and confers the characteristic transparency of these eggs (de Vlaming, 1983; Selman and Wallace, 1989).

Bromage and Cumaranatunga (1988) and Selman and Wallace (1989) consider that within the teleosts, the accumulation of the nutrient reserves required for future embryonic and early larval development essentially involves four phases:

1. Synthesis and secretion by the liver of the yolk precursor material called vitellogenin.
2. Transportation of vitellogenin in the circulatory system to the developing oocytes.
3. Sequestering of vitellogenin by receptor-mediated endocytosis at the surface of the oocyte.
4. Translocation of vitellogenin to yolk bodies within the ooplasm and its proteolytic cleavage into the yolk constituents phosvitin and lipovitellin.

Hormonal control of reproductive development will be discussed in chapter 5, however it is pertinent at this stage to accept that vitellogenin synthesis and export by

the liver is stimulated by steriod hormones, in particular 17β-estradiol, synthesized by the follicle surrounding the developing oocyte (Mommsen and Walsh, 1988).

3.4.5.4 Vitellogenin.

The hepatically synthesized vitellogenin molecule consists of a protein chain (M.W. 250,000 - 600,000) together with substantial amounts of carbohydrate, lipid material, phosphate groups and mineral salts (Mommsen and Walsh, 1988). De Vlaming (1983) describes vitellogenin as a female-specific serum phospholipoglycoprotein while Gillis *et al.* (1990) describe the molecule as an alkali-labile phoshorylated protein. It is apparent that vitellogenin molecules present in teleosts show a high variability in biochemical parameters (molecular weight, degree of phoshorylation and lipidation, subunit composition) between species and even within the same species. It is suggested that this variability is due to vitellogenin being encoded by a number of closely related genes (Mommsen and Walsh, 1988; Selman and Wallace, 1989).

It is generally accepted that blood borne vitellogenin synthesized by the liver is the precursor to the protein yolk material of fish oocytes. Many studies on different aspects of the processes operating during vitellogenesis now support this accepted scheme. The inter-relation between the liver and proteid yolk accumulation in developing oocytes was shown in an autoradiographic study conducted by Korfsmeier (1966). Results showed that tritiated amino acids injected into continuously breeding zebrafish, *Branchydanio rerio*, first appeared as labelled protein in the liver and subsequently in the peripheral yolk globules of the developing oocytes. A number of studies have identified and characterised highly specific estrogen binding proteins (receptors) in hepatic tissue of fish (Mommsen and Walsh, 1988). mRNA for vitellogenin has been isolated from the liver of some teleosts (Guraya, 1986). Korsgard *et al.* (1986) report a greater than 30% increase in cellular RNA in response to estradiol treatment of Atlantic salmon, *Salmo salar*, and suggest that the de novo synthesis of mRNA for vitellogenin may account for some of this increase.

Aida *et al.* (1973a.) using starch gel electrophoresis and immunoelectrophoresis revealed the existence of two female specific plasma proteins (FSPP) induced in the plasma of female ayu, *Plecoglossus altivelis*, following estrogen administration. FSPP could also be induced in male fish following administration of 17β-estradiol. The authors concluded that FSPP were formed outside the ovary following stimulation with estrogen and appear in the plasma, from were they are taken up by the oocyte during vitellogenesis. Following on from this work Aida *et al.* (1973b.) investigated the histological changes in liver cells (hepatocytes) following estrogen administration using the same species. Hepatosomatic index (liver weight as a percentage of total fish weight including gonads) increased following administration of 17β- estradiol. Light microscopy showed that glycogen and lipid content declined markedly and binucleate cells were frequently seen suggesting a hyper-function of nucleus and nucleolus. Liver cell nuclei of treated fish became hypertrophied with nuclei containing 1-3 enlarged nucleoli. High concentrations of RNA in the periphery of cells was indicated by strong staining of this basophilic material using pyronin. Electron microscopy revealed more dramatic changes, showing a remarkable proliferation of rough endoplasmic reticulum (rER) and golgi bodies containing electron dense material. This proliferation of cytoplasmic organelles is suggested to be indicative of increased protein (vitellogenin) synthesis.

Recently Gillis *et al.* (1990) have shown a hepatic source for vitellogenin in the Pacific herring, *Clupea harengus pallasi*. In their study the hepatosomatic index for females was higher than that of males and declined during population ovulation. In their electronmicroscopic examinations hepatocytes were designated as electron-lucent, showing ultrastructural features indicative of vitellogenin synthesis, or electron dense, with features not indicative of protein synthesis. Electron lucent hepatocytes dominated in livers of females during late vitellogenesis displaying ultrastructural features supporting their function as sites for vitellogenin synthesis. These features included the presence of abundant dilated rER and large secretory vacuoles associated

with large well developed Golgi bodies. Lucent hepatocytes were scarce in males during all stages examined and were less abundant in ovulated females.
It can be concluded from these studies that, in response to endogenous or exogenous (administered) estrogen, hepatocytes and their cytoplasmic organelles proliferate resulting in an increased capacity for vitellogenin synthesis. The accompanying decrease in hepatosomatic index during maturation supports this explanation if it is accepted that the glycogen and lipid are consumed in the production of this lipoglycoprotein. Further examples of the cellular events induced by estrogen are provided in Table 3.1.

Formation of vitellogenin in the liver commences with the synthesis of the protein backbone on membrane bound ribosomes. The molecule is subsequently lipidated, glycosylated and phosphorylated prior to packaging into golgi bodies and secretion into the bloodstream. Vitellogenin carries a number of phosphate groups in the region that will ultimately be deposited as phosvitin in the mature oocyte, although this amount is only about 50% of the protein phosphate content of other oviparous vertebrates. This alkaline-labile protein phosphorous can be readily used to determine the degree of vitellogenic response in fish. The amount of lipid material carried by the vitellogenin molecule is about twice that carried in other vertebrates accounting for around 20% of the weight of the molecule. Most of this lipid material is classified as polar lipid which ultimately contributes the lipovitellin moiety of the yolk. The mature oocyte also contains significant amounts of non-polar lipids such as triglycerides, steryl esters, sterols, and wax esters which are assumed to be derived from sources other than vitellogenesis (Mommsen and Walsh, 1988).

3.4.5.5 Yolk globule formation.

Hepatic golgi bodies secrete vitellogenin into the plasma for delivery to the developing oocytes in the circulatory system (Mommsen and Walsh, 1988). The mechanics accompanying the uptake of vitellogenin by the developing oocyte is subject to some

Transient decrease in cytosolic estrogen receptor protein
Induction of nuclear estrogen receptor protein
Increase in hepatosomatic index due to hyperplasia or hypertrophy
Proliferation of Golgi apparatus
Increase in cisternae of the nuclear envelope
Synthesis of ribosomes
Polysome assembly
Increase in rough endoplasmatic reticulum
Swelling of mitochondria
Appearance of a new species of mRNA (vitellogenin)
Increase in protein synthetic activity
Synthesis of vitellogenin
Increase in cellular RNA
Increase in lipid metabolism (?)[a]
Augmented output of very low density lipoproteins (VLDL)
Decrease in glycogen content per cell
Increase in metabolic enzymes
Higher amount of hepatic DNA
Increase in hepatic water content

[a] For the fishes, to date only circumstantial evidence suggests this particular alteration.

Table 3.1 Cellular events induced by estrogen in hepatic tissue of teleosts. (From Mommsen and Walsh, 1988).

disagreement. Extensive micropinocytotic activity is characteristic of the surface of vitellogenic oocytes and highly selective uptake of vitellogenesis has been suggested (Wallace and Selman, 1981). In a more recent review (Selman and Wallace, 1989) the same authors state that the selective uptake of vitellogenin is an area requiring further investigation a view shared by Mommsen and Walsh (1988). It is suggested that vitellogenin binds to an oocyte surface receptor, a process involving the phosphorylation or glycosylation of the vitellogenin molecule to allow recognition. Subsequently the vitellogenin molecule is proteolytically cleaved into lipovitellins, phosvitins and phosvettes during its translocation from the oocyte surface to the yolk deposition sites. Receptor molecules are recycled in this process while the yolk components are deposited in membrane bound, fluid yolk globules (Mommsen and Walsh, 1988). Selman and Wallace (1989), who summarised recent descriptive studies, implicate the involvement of lysosome-like, multivesicular bodies in the

processing of vitellogenin within oocytes. These structures are prominent at the start of vitellogenesis and disappear with the accumulation of yolk globules.

3.4.6 Oocyte maturation and ovulation.

When oocytes have attained a species specific size, vitellogenesis stops and the oocytes undergo a number of nuclear and cytoplasmic processes accompanying their final maturation and ovulation, as directed by appropriate hormonal stimulation (chapter 5). These processes include the peripheral migration and dissolution of the germinal vesicle (nucleus) membrane (GVBD), resumption of meiosis with accompanying condensation of chromosomes, and extrusion of the first polar body (Wallace and Selman, 1981; De Vlaming, 1983; Goetz, 1983; Guraya, 1986). Maturation is a rapid process in most teleosts, generally being accomplished within 24 hours (Selman and Wallace, 1989).

Commencement of maturation is indicated by the migration of the nucleus towards the micropyle to distinguish the animal pole of the maturing oocyte (Guraya, 1986). Meiosis resumes at GVBD following the dissolution of the germinal vesicle membrane so that nuclear contents mix with the surrounding ooplasm (de Vlaming, 1983; Goetz, 1983; Guraya, 1986). Chromosomes condense and meiosis proceeds to first metaphase and the first polar body is released. Meiosis continues until becoming arrested again at second metaphase (De Vlaming, 1983; Guraya, 1986; Selman and Wallace, 1989). Arrested at this stage of development the oocyte is recognised as being fertilizable and is thus "mature" (Selman and Wallace, 1989).

As these events take place changes occur in the cytoplasm, where a separate coalesence of yolk globules (proteid yolk) and lipid droplets (lipid yolk) results in the characteristic transparency of many teleost eggs. Accompanying these changes is a further rapid increase in size due to hydration, which is particularly pronounced in oocytes of marine teleosts (De Vlaming, 1983; Goetz, 1983; Guraya, 1986; West,

1990). Uptake of water due to hydration can increase oocyte volume by 300-400% in some species (De Vlaming, 1983) and can represent up to 86 % of the final size of the egg (Selman and Wallace, 1989).

Selman and Wallace (1989) summarise recent findings on the mechanisms of the hydration process by which such eggs become buoyant in sea water. During oocyte maturation there is an accumulation of K^+ and an increase in protein dephosphorylation, which may together, or in isolation, contribute to the osmotic effects involved in the process of hydration. Craik and Harvey (1987) studied the causes of buoyancy in eggs of marine teleosts. They suggest that the influx of water during oocyte maturation can be explained as a consequence of the breakdown of yolk protein into small free amino acid molecules which causes an influx of potassium and sodium ions from the blood plasma.The resulting increase in small molecules and ions attracts an accumulation of water from the plasma sufficient to maintain osmotic equilibrium between yolk and blood plasma. These authors found that both demersal and pelagic eggs have osmotic concentrations similar to that of maternal body fluids which is less than half that of sea water. When pelagic eggs are released into sea water this high content of dilute fluid, and not the lipid content, causes buoyancy.

Either towards the end of the process of maturation, or soon after its completion, the oocyte is usually ovulated into the ovarian lumen, or peritoneal cavity. At this time oocytes have completed the first meiotic division and are arrested at the second metaphase and can consequently be refered to as eggs. (Wallace and Selman, 1981; De Vlaming,1983; Guraya, 1986; Selman and Wallace, 1989). Expulsion of the egg is achieved by contraction of the follicular wall (de Vlaming, 1983; Guraya, 1986). Oshiro and Hibiya (1982) suggest that prior to this, proteolytic enzymes are involved in disrupting the association of the follicle and oocyte.

3.5 Fertilization.

A comprehensive account of the mechanisms of fertilization in fishes is presented by Gilkey (1981). This author describes three categories of sperm-egg interacting substances which function to unite mature eggs and sperm. These substances operate in the order in which they are presented, namely; modulators of sperm-motility, chemotactic agents and membrane-bound complementary receptors.

Modulators of sperm-motility function to inhibit or enhance sperm motility. Sperm of teleosts is generally immotile before release; consequently, in order to reach the egg it must become motile. It is suggested that high potassium, or a low sodium to potassium ratio of the epididymal suspension may act as the inhibitor to sperm motility. Motility is initiated when sperm are released into a media which dilutes these conditions (Rothschild, 1958).

Initiation or enhancement of sperm motility is also achieved by chemotactic substances which can be extracted from the micropylar region of the egg. Immotile sperm of *Clupea harengus pallasi*, have been shown to become become active and swim vigorously when in close contact with the egg chorion. This response is produced by isolated chorionic pieces with a micropyle (Laale, 1980). Gilkey (1981) postulates that the eggs and sperm of oviparous fishes are released in close association which may make the chance of encounter sufficiently high to obviate the need for chemotaxis as a mechanism to unite mature sperm and eggs. It is suggested that if this were the case, the chemotactic substances associated with the micropyle would function merely to direct the sperm to the micropyle.

In teleosts, membrane-bound complementary receptors may exist in the sperm and on the egg-plasma membranes which are directly accessible to sperm, through the micropyle. It is thought that these receptors may function to permit species-specific

binding of sperm and homologous eggs. They may also assist in the fusion of sperm and egg, or they may aid the interpretation of sperm-egg contact to provide an activating stimulus for the egg (Gilkey, 1981).

At fertilization the mature oocyte is arrested at second metaphase of meiosis as previously described. Fertilization of the egg by a sperm initiates a complex sequence of events, collectively refered to as activation. These events result in the resumption of meiosis and allow further development to proceed. During fertilization the sperm crosses the chorion through the micropyle and the sperm head is enveloped by a "fertilization cone" formed by lobopodial extensions of the plasma membrane. Fusion occurs, with the subsequent incorporation of the head of the sperm into the cytoplasm. An increase in the concentration of free calcium in the egg cytoplasm following fertilization is thought to be the activator for further egg development (Gilkey, 1981).

Gilkey (1981) conducted research on egg activation using the photoprotein aequorin, which is dependant on free calcium for luminescence. Luminescence of aequorin loaded eggs of medaka, *Oryzias latipes*, was followed using an image intensifier to visualise a "calcium wave", initiated by fertilization. This phenomenon starts beneath the micropyle and crosses the egg as a narrow band before closing on itself at the antipode. It is believed that the calcium wave is propagated through the thin peripheral layer of cytoplasm by a calcium dependant release of calcium from local stores, likely to be cortical endoplasmic reticulum. Sperm induces a local rise in the free calcium to a threshold level which stimulates the release of calcium from these stores to propagate the front of the wave. Following activation the developmental events of the egg proceed.

After fertilization the perivitelline space is formed. This forms a liquid cushion between the egg and its membranes, serving a protective function (Laale, 1980). Laale (1980) concludes that the perivitelline space forms as a result of the separation of the

egg envelope from the egg proper caused by an osmotic distension of the egg membrane and the shrinkage of the egg away from this membrane. Egg shrinkage is attributed to fertilization-activated discharge of egg substance (polysaccharide derived colloid), from the cortical alveoli, into the developing perivitelline space. Membrane elevation is due to absorption of water and electrolytes drawn into the perivitelline space by the osmotic pressure initiated by these colloids during this "cortical reaction". Morrison (1990) provides a good description of fertilization for Atlantic cod, *Gadus morhua*, stating that the perivitelline space forms as a wave from the micropyle and all cortical alveoli disappear within 10-20 minutes following fertilization. Although a small perivitelline space is formed in unfertilized eggs of *Gadus morhua*, cortical alveoli persist.

3.6 The follicle.

The follicle wall of teleosts is made up of the chorion of the oocyte and an inner granulosa layer separated from an outer thecal layer by a basement membrane (Kagawa *et al.*, 1982; De Vlaming, 1983). The chorion (vitelline membrane, zona radiata, zona pellucida, primary envelope) starts to form between the oocyte and the granulosa cells during the perinucleolus stage of oocyte growth. At this stage, electron dense material begins accumulating between microvilli projecting from the surface of the oocyte, towards the follicle cells. Material accrues extracellularly and the chorion continues to differentiate as the oocyte grows (Selman and Wallace, 1989). Both the oocyte and/or the granulosa cells have been attributed as being responsible for the synthesis of the chorion and the resolution of this question requires further investigation (Laale,1980; Selman and Wallace, 1989). The chorion is is a multilayered structure in *Gadus morhua*, with an outer layer, an intermediate homogeneous layer and an inner, multilamellar layer (Kjesbu and Kryvi, 1989)

Selman and Wallace (1989) report that in the pipefish, *Syngnathus scovelli*, the oocyte becomes enveloped by a layer of follicle cells before leaving the germinal ridge. These granulosa cells are in contact with the oocyte during primary stages (chromatin nucleolar, perinucleolar) of development and their number increases as the oocyte grows, probably by mitosis, retaining a single layered structure throughout oocyte development (De Vlaming, 1983; Guraya, 1986).

Based on ultrastructural and histochemical studies, granulosa cells have been attributed a number of functions during oocyte growth including, the synthesis of proteins and lipids and steroidogenesis (Guraya, 1986). During oocyte development microvillar projections from the oocyte and cytoplasmic projection from the follicle cells, penetrate the chorion to variable distances depending on the stage of oocyte growth. These cellular projections become housed in pore canals traversing the chorion and this association allows communication between the oocyte and granulosa cells. Proteins and lipid bodies built up in the granulosa cells seem to be able to be transported into the ooplasm of the developing oocyte. The transport mechanism for this material probably involves diffusion, active transport and pinocytosis (De Vlaming, 1983; Guraya, 1986; Selman and Wallace, 1989).

Between the granulosa and thecal layers of the ovarian follicle is the basement membrane (basal lamina), consisting of a series of membranes containing collagen fibres and electron dense material (Guraya, 1986). The thick thecal layer contains collagen fibres, capillaries and fibroblast-like cells, some of which are identified as enlarged, special thecal cells (De Vlaming, 1983; Guraya, 1986). It has been established beyond doubt that the ovarian follicle of teleosts is capable of synthesizing estrogens, progesterogens, androgens, and restricted groups of corticosteroids. It is generally accepted also, that pituitary gonadotrophin acts on the follicle to stimulate production of 17β-estradiol (Kagawa *et al.*, 1982). The ovarian follicle layers (granulosa and theca) are believed to be the major sites of this steroidogenesis

however the contribution of the separate follicular components is still to be elucidated completely (Lance and Callard, 1978; Guraya, 1986).

The involvement of the granulosa cells in steroidogenesis is divergent. Ultrastructural studies suggest that the granulosa cells do not show convincing evidence of steroidogenesis, and histochemical studies have presented conflicting results in regard to the role of these cells in the synthesis of 17β-estradiol (Nagahama *et al.*, 1976; Guraya, 1986). The hypertrophied special thecal cells are located close to capillaries and display ultrastructural features indicative of a role in steroid synthesis. This suggests that they are the major cellular site for steroidogenesis in the teleost ovary (Nagahama *et al.*, 1976; Guraya, 1986). The endocrine role of the follicle will be expanded upon in chapter 5.

Post-ovulatory follicles composed of remnant granulosa and thecal layers (Bromage and Cumaranatunga, 1988) remain for relatively short periods following spawning (De Vlaming, 1983). Some confusion exists relating to the possible steroid hormone biosynthetic capacity of post-ovulatory follicles. De Vlaming (1983) is of the opinion that the limited data available implies a steriod synthetic capacity. Nagahama *et al.* (1976) state that in goldfish, *Carassius auratus*, post ovulatory follicles 6-10 hours after ovulation show a highly vascularised thecal layer and hypertrophied granulosa cells. Special thecal cells and granulosa cells show weak but evident 3β-hydroxy steroid dehydrogenase (3β-HSD) activity indicating a steroidogenic capacity. Both cell types showed advanced degeneration 30 hours after ovulation which suggests that post ovulatory follicles do not have an endocrine function at this stage.

3.7 The process of atresia.

Follicular atresia (degeneration) is a common process in the ovaries of vertebrates. In this process oocytes at various stages of development are removed from the ovary

(Saidapur, 1978; Nagahama, 1983; Guraya, 1986). Some disagreement exists regarding the extent of atresia of oocytes prior to ovulation. Bromage and Cumaranatunga (1988) argue that only atresia can account for the progressive reduction of pre-vitellogenic oocytes of rainbow trout *Oncorhynchus mykiss* , from 15,000 per 100 g of body weight prior to the start of exogenous vitellogenesis, leaving only 200 mature oocytes per 100 g body weight at ovulation. These authors state that between 10-30% of vitellogenic oocytes may be atretic at any one occasion and suggest that atresia of smaller pre-vitellogenic oocytes may be extremely rapid. Alternatively, De Vlaming (1983) and Guraya (1986) do not quantify oocytes but they cotend that during normal ovarian recrudescence (pre-spawning) in teleosts a relatively small percentage of oocytes become atretic. Pacific herring, *Clupea harengus pallasi*, also develop more eggs than are matured with selective resorption accounting for the decrease in numbers of maturing oocytes (Hay, 1985). In situations of adverse environmental conditions authors agree that an entire clutch of oocytes may become atretic (De Vlaming, 1983; Bromage and Cumaranatunga, 1988) however such pre-spawning atresia or "derecruitment", is uncommon in healthy (non-stressed, well-fed) females (Wallace and Selman, 1981).

Following oocyte development it is also common that a small number of vitellogenic oocytes will not be ovulated before spawning and and some hydrated oocytes will also remain to be reabsorbed during the post-spawning recovery process (Guraya, 1986; Morrison, 1990). These residual eggs are generally removed by undergoing the process of atresia, the final stages of which are refered to as "brown bodies" (Bromage and Cumaranatunga, 1988). The atresia of these vitellogenic follicles appears to follow a similar pattern in all species of fish studied (Saidapur, 1978) and can be regarded essentially as a "mopping up" process (Wallace and Selman, 1981). Granulosa cells of the follicle hypertrophy and become phagocytic. This enables them to digest and dispose of the yolk remnants of the ovum. During this process the granulosa cells proliferate and together with thecal cells and blood vessels, may

invade the follicular space. The disintegration of these structures results in an irregular clump of cells (Saidapur, 1978). A thorough description of atresia observed in rainbow trout is presented by Bromage and Cumaranatunga (1988).

Guraya (1986) concludes from morphological and histochemical information, that atretic vitellogenic follicles do not have an endocrine role, while Morrison (1990) states that a hormonal function has been suggested for atretic eggs. Resorption of atretic oocytes have not been shown to affect subsequent oogenesis in the bream, *Abramis brama*, or the white sucker, *Catostomus commersoni* (Trippel and Harvey, 1990).

Ovaries of spent fish are generally relatively large and flabby with a thickened ovarian wall, *tunica albuginea* . Morrison (1990) states that ovaries of Atlantic cod, *Gadus morhua*, which have completely resorbed atretic oocytes and have started vesicle formation, are firmer than those which still contain atretic oocytes. Observations of ovaries of *Latris lineata* are consistent with this description and support the notion that oocyte resorption is completed prior to cortical alveoli formation (section 4.5).

3.8 Classification of developing gonads.

Within oviparous teleosts gonad development is characterised by a series of events common to most species, with the greatest diversity occurring in recruitment and timing of these events (De Vlaming, 1983; Wallace and Selman, 1981). Many authors (Table 3.2) provide classification guidelines which attempt to correlate external appearance of the gonads (visual assessment, macroscopic staging) with the stages of reproductive development occurring at a cellular level (histological assessment, microscopic staging). The nett situation existing appears confusing, given the commonalities inherent in the processes of oogenesis and spermatogenesis occuring within the teleosts collectively.

Author	Year	Genus and species	Common name	No. Stages
Bromage and Cumaranatunga	1988	*Oncorhynchus mykiss*	Rainbow trout	7
Hoffman and Grau	1989	*Thalossoma duperrey*	Hawaiian saddleback wrasse	3
Hay	1985	*Clupea harengus pallasi*	Pacific Herring	8
Kjesbu	1991	*Gadus morhua* L.	North-east arctic cod	6
Young *et al.*	1987	*Lampanyctodes hectoris*	Two lantern fish species	Females (F) 6
		Diaphus danae		Males (M)
		Maurolicus muelleri	Pennant lightfish	5
Davis	1982	*Lates calcarifer*	Barramundi/Sea bass	6
Munehara and Shimazaki	1989	*Hexagrammos octogrammus*	Masked greenling	6
Asano and Tanaka	1989	*Scomber japonicus*	Japanese common (chub) mackeral	8 plus 3 sub-classes
Gunn *et al.*	1989	*Macruronus novaezelandiae*	Blue grenadier	7
Abu-Hakima	1984	*Acanthopagrus latus*	Yellow-finned black porgy	8
		A. cuvieri	Silvery black porgy	
Pankhurst *et al.*	1987	*Hoplostethus atlanticus*	Orange roughy	F = 6 M = 5
		Pseudocyttus maculatus	Smooth oreo dory	
		Allocyttus sp.	Black oreo dory	

Table 3.2 Number of maturity stages used by selected authors as determined by macroscopic assessment of gonads.

West (1990) concludes from an unpublished CSIRO study on *Lutjanus vittus*, that the accuracy of macroscopic staging varies for different stages of oocyte development. It is recommended that ovaries should be staged based on the appearance of the most advanced oocytes when viewed using a stereomicroscope. Although macroscopic staging is accurate for distinguishing unyolked, yolked and hydrated oocytes, the technique cannot accurately differentiate between yolked and ripe oocytes (vitellogenesis completed, approaching migratory nucleus stage). The same author states that histology is the most accurate method to determine the reproductive state of females, and is of the opinion that macroscopic assessment should be related to the stage of development as determined by a histological study. When such a methodology is employed, the qualitative descriptions required for macroscopic assessment can be avoided and the reproductive stage can be aligned to the features of oocyte development occurring at the cellular level.

3.9 Dynamic aspects of oocyte development in teleosts.

Within the teleosts there is considerable diversity regarding the duration of the stages comprising oocyte development, ultimately reflected as a diversity in spawning patterns between the many different species. Such variation in ovarian organisation represents the different strategies adopted by individual species, to assure them of the best possible reproductive success in their environment (Wallace and Selman, 1981). Within the teleosts three basic patterns of ovarian organisation are identified (Wallace and Selman, 1981; De Vlaming, 1983). These are as follows;

1 Synchronous oocyte development.

Once formed, all oocytes develop and are ovulated from the ovary at the same time, without further recruitment. Teleosts which spawn once and then die (i.e. Pacific salmon, *Oncorhynchus* species, catadromous eels) display this pattern of oocyte development (Wallace and Selman, 1981).

2 Group-synchronous oocyte development.

In this pattern of development at least two populations (clutches) of oocytes are present and the spawning season may be protracted. The largest clutch of oocytes is more uniform in diameter than the smaller more heterogeneous oocytes from which the larger clutch is recruited. Group-synchronous oocyte development has been found to be the most common ovarian type among the teleosts. Different numbers of clutches and frequencies of recruitment are found between species displaying group-synchronous ovaries (Wallace and Selman, 1981; De Vlaming, 1983, West, 1990). The recruitment of clutches of oocytes is thought to be initiated by the gonadotrophin surge accompanying the onset of final oocyte maturation, so that as a clutch enters maturation another clutch is recruited into vitellogenesis. Multiple clutches can develop in this manner with successive clutches progressing to different stages of vitellogenesis as recruitment occurs (Wallace and Selman, 1981).

3 Asynchronous oocyte development.

Asynchronous oocyte development is a feature of some species of teleosts having long spawning seasons with multiple spawnings. Ovaries of these species display a wide range of oocyte diameters so that pronounced clutches are not found. It is suggested that in asynchronous ovaries, there is a continuous recruitment of oocytes into vitellogenesis and a subsequent periodic recruitment into final oocyte maturation (De Vlaming, 1983).

4 THE REPRODUCTIVE BIOLOGY OF *Latris lineata*.

4.1 Collection and preservation of gonad samples.

The reproductive biology of *Latris lineata* has not been previously documented, a situation which is disadvantageous for persons wishing to develop methods to control egg production from this species. In order to record the reproductive development of *Latris lineata*, sampling of gonads obtained during commercial fishing operations, was undertaken on 10 occasions during this study between 25 May 1990 and 22 May 1992. The ideal objective of this component of the project was to collect gonad samples at monthly intervals. In reality this was not able to be achieved due to the combined limitations introduced by the logistics of coordinating with commercial fishermen, themselves dictated by the vagaries of Tasmanian weather, and other research undertaken at the times (spawning induction and larval rearing) approaching, during and following the annual spawning season. The results presented, although not ideal, serve the purpose of providing base-line information to assist further research into the controlled spawning of *Latris lineata*.

4.1.1 Fishing operations.

The early stages of this project focused on making contact with commercial fishermen regularly fishing for Striped trumpeter, who would allow sample collection during fishing operations. An unsuccessful four-day trip to the Flinders Island and Clark Island in north-east Tasmania was followed by another unsuccessful four-day trip to the waters outside of Port Arthur in south east Tasmania. On both occasions weather conditions did not allow fishing for Striped trumpeter to be attempted. After these initial failures contact was made with two fishermen Mr. Noel Harper, working out of St. Helens and Mr. Eddy Freeman working out of Port Arthur, both of whom were extremely helpful and supportive of this project.

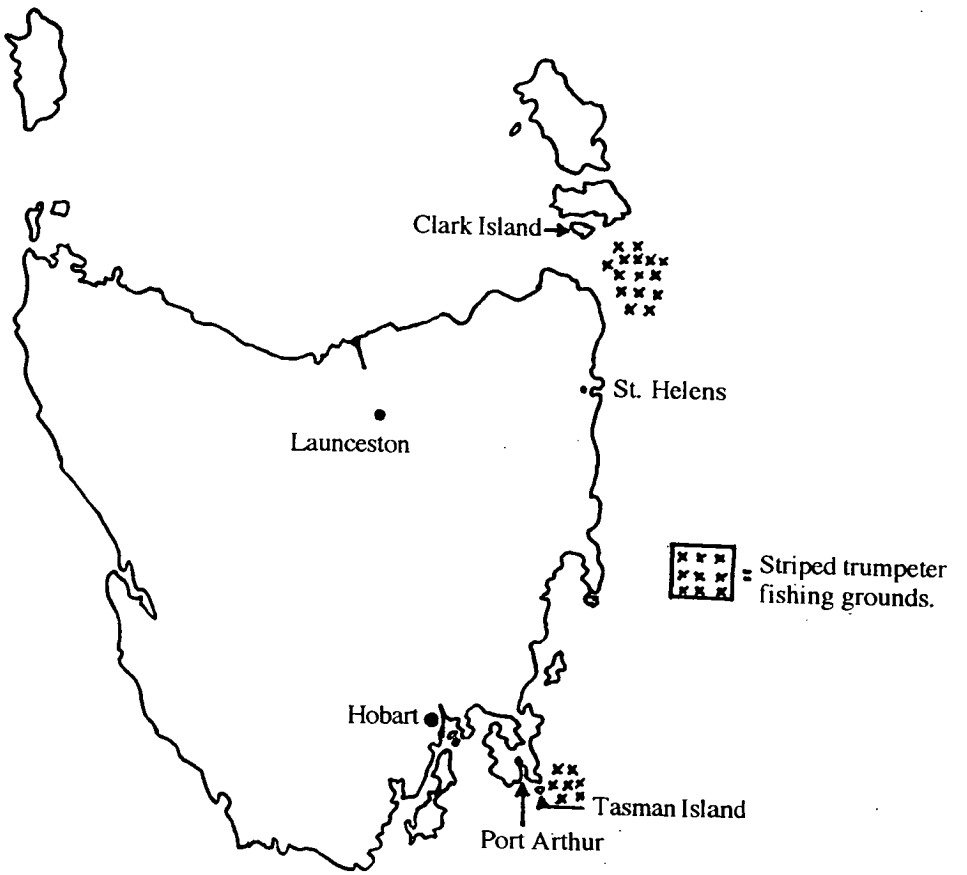


Figure 4.1 Location of fishing grounds from which *Latris lineata* were captured during this study.

For reasons of convenience, most sampling (8 of the 10 sampling dates) was undertaken from St. Helens where all but 11 fish were caught in the same area, north east of St.Helens and south east of Clark Island. Fish sampled from Port Arthur were caught in the vicinity of Tasman Island (Figure 4.1). Fishing operations were conducted over hard bottoms at depths ranging from 70 to 90 fathoms (140-180 m) as identified by a colour depth sounder. Past fishing areas were relocated using global positioning satellite (GPS) equipment. The method of capture essentially was by line with a variety of gear configurations used as fishermen developed methods appropriate to suit best the operation of their boat (Figures 4.2, 4.3 and 4.4).

When using droplines (Figure 4.2) each line (4-5 individual lines used) was prepared with 20 heavy guage 8.0 size hooks, each set on a 150-200 mm long, 4 mm nylon cord snood. Hooks were pre-baited with squid, octopus or jack mackeral (*Trachurus declivis*) and attached with stainless steel shark clips, at 30-40 cm intervals, to the bottom 7-8 m of the nylon rope (8 mm) dropline. The gear was deployed over locations where schools of fish were identified and was retrieved at approximately 30 minute intervals. Fish were removed by detaching the shark clip from the dropline, new baits were attached and the line then redeployed.

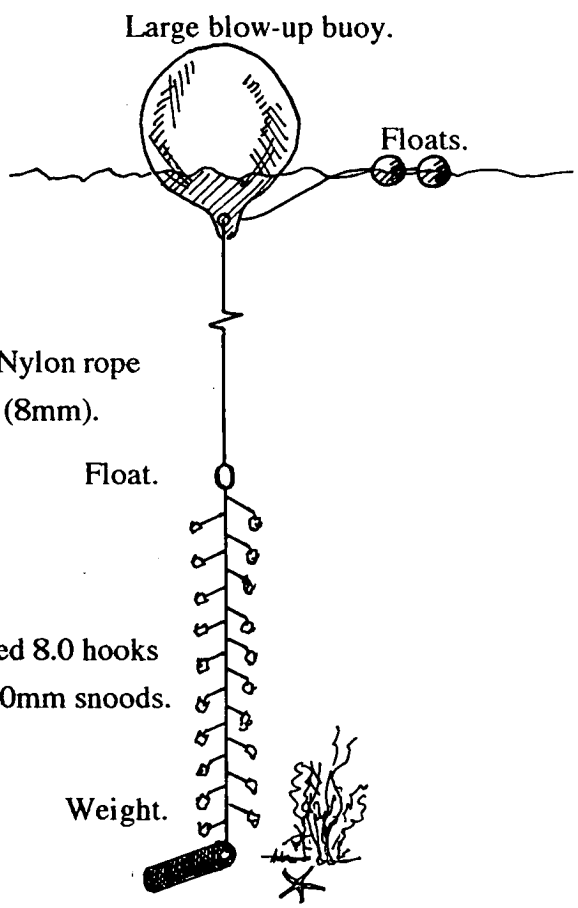


Figure 4.2 Arrangement of drop-line used to capture *Latris lineata.*

Hand-lining for striped trumpeter was undertaken using detachable Alvey hand winches (Figure 4.3) fitted with 100 kg breaking strain monofilament line. The boat was anchored at a position to allow fishing to take place over identified locations. Three winches were used with each operator bringing up one or more fish as they were hooked.

Figure 4.3 Hand operated winch (hand-line) used to capture *Latris lineata*.

The trot-line method (Figure 4.4) used similar gear to that used in the dropline method however the hooks were presented on solid 20 mm round plastic rods. Each 2 m rod had 8 hooks attached with a float at one end and a shark clip at the other connecting to a bottom line of nylon rope. As the gear was deployed, 20-30 poles were clipped on to the bottom line at 5-6m spacings. Gear was set over identified hard bottom and secured with an anchor at each end of the line of poles. After 20-30 minutes the gear was retrieved and all poles were detached as the bottom line was winched on board. Fish were removed from the poles on deck and released into a holding well before rebaited poles were attached in order that the operation could be repeated.

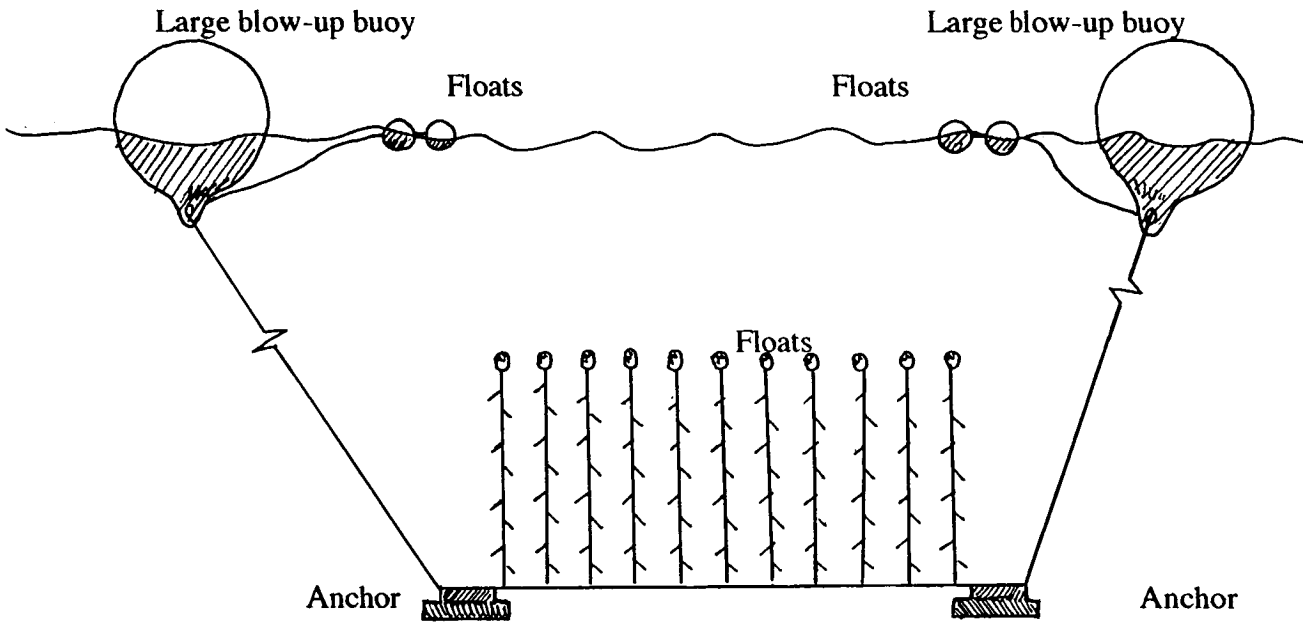


Figure 4.4 Arrangement of trot-line used to capture *Latris lineata*.

4.1.2 Sample collection and preservation.

Sampling procedures were conducted as fish were being processed at sea, or at port on those occasions when fish were maintained alive until processing on the day after capture. On these occasions fish were weighed to the nearest 20 g using a top pan balance (Wedderburn, 12 kg capacity). The sex and fork length of each fish was recorded and the gonad dissected from the body cavity (Figure 4.5) and placed in an appropriately labelled plastic bag which was immediately transfered into ice. Upon return to Launceston that evening, each sample was removed from ice storage, attached fat and membranes were cut away and the weight of the paired gonad measured to the nearest 0.1 g using an Sartorius PT 1200 portable electronic balance. Photographs of representative gonads were taken then each sample was transfered into approximately 4 times their volume of formol calcium (10% formaldehyde with 2 % calcium acetate in distilled water) for preservation and storage prior to processing for histological analysis. For large gonads only a cross section (10-20mm) of tissue was taken from the central region of the organ to allow adequate tissue preservation.

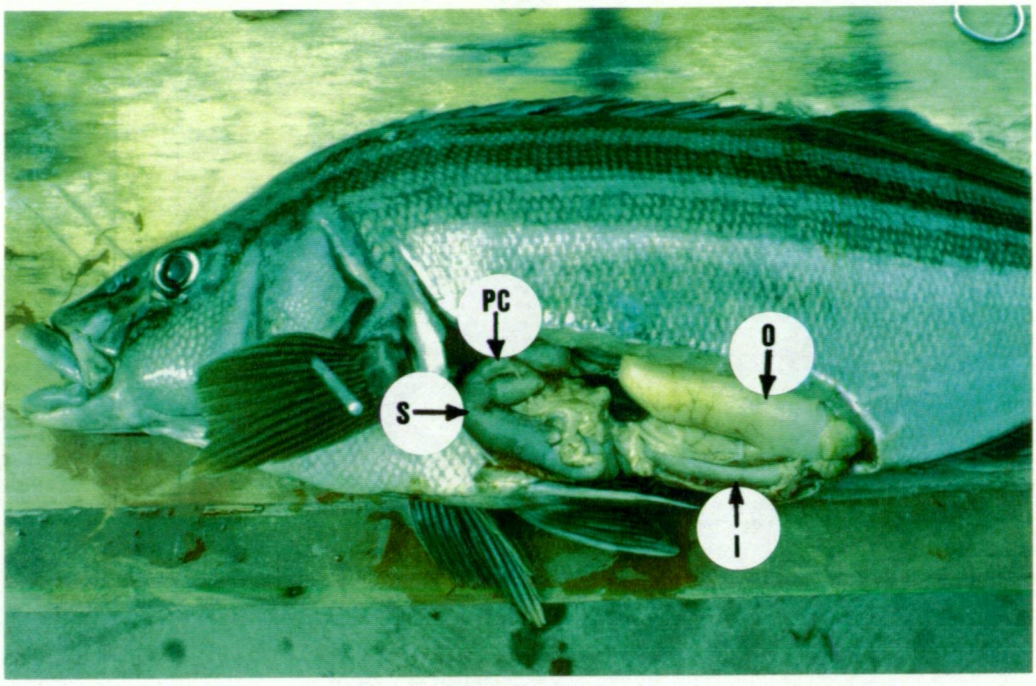


Figure 4.5 Location of ovaries (and testis in male fish) in *Latris lineata*.
O = Ovary; S = Stomach; PC = Pyloric caeca; I = Intestine (Hind-gut).

4.2 Processing and analysis of gonad samples.

4.2.1 Histological methods.

Following a 30-day hardening period the gonads were removed from the preservative solution within a fume cabinet. A 2-3mm cross section was dissected from the central region of one of the paired gonads and placed in an appropriately labelled Tissue-tek histological cassette and transfered into clean formal calcium. All gonad samples were processed using a Tissue-tek II tissue processor on a 24 hour cycle. Samples were subsequently embedded in parafin wax using a Shandon Histocentre 2. Wax-embedded samples were sectioned to 4μm with a Microm HM 340 microtome after which they were stained with heamatoxylin and eosin in a Shandon Linistain GLX staining machine.

4.2.2 Oocyte measurement and analysis procedures.

It is recognised that preservation and processing procedures will alter the dimensions of oocytes due to shrinkage. West (1990) provides a review of the effects of preservation and processing of teleost oocytes. No shrinkage factor was determined in this study so that all values reported refer to those measured from histologically-prepared samples.

A CUE - 2 image analysis system was used to measure oocytes. Histological slides prepared from gonad cross sections were examined on an Olympus BH-2 stage microscope with a CCE video camera fitted to allow visualisation on a Sony Trinitron monitor. Working systematically from top to bottom of each slide in horizontal passes, sectioned oocytes were selected for analyses based on the the following criteria;

1 Oolemma complete/unbroken

2 Germinal vesicle centrally located.

3 Large amount of the germinal vesicle present in oocytes prior to germinal vesicle breakdown.

4 Largest oocytes plus other oocytes fulfilling these criteria which were within the frame aquired on the monitor.

The image analysis system allowed selected oocytes to be highlighted and their cross sectional area measured, from which the mean diameter of each oocyte was automatically computed. A suitable number of oocytes were measured from each frame according to the stage of development and the number of large oocytes present. Typically 2-6 oocytes were measured from each frame aquired for analysis. 50 representative oocytes were measured for each sample although 30 oocytes were measured for some pre-vitellogenic samples with a uniform range of oocyte diameters.

4.3 Microscopic description of reproductive development in *Latris lineata.*

In order to build a picture of the pattern of reproduction followed by *Latris lineata*, it is important to document the stages of gonad development as they occur in this species. Histological studies are recognised as providing the most accurate information on gonad development (West, 1990). The objective of conducting a histological investigation of gonads sampled in this study was to provide a description of the events occurring at the cellular level from which, simpler less time-consuming methods (i.e. macroscopic/visual assessment) of reporting gonad development in *Latris lineata*, could be substantiated.

4.3.1 Histological description of the stages of oocyte development in *Latris lineata.*

4.3.1.1 Primary stages of oocyte growth.

Primary stages of oocyte growth include the chromatin nucleolus and subsequent peri-nucleolus stages which are found in both immature and mature female fish as primary oocyte growth occurs independent of pituitary influence (Wallace and Selman, 1981). Oocytes of various stages of primary growth can be seen within the ovigorous folds of *Latris lineata*. The chromatin nucleolus stage oocytes seen in Figures 4.4a and 4.4b are variable in shape, characterised by a central nucleus and a single deeply-staining basophilic nucleolus. Chromatin nucleolus stage oocytes of *Latris lineata* display a size range between 40-100 μm with a nucleus which appears large in comparison to the small amount of surrounding cytoplasm. In *Latris lineata*, germinal cells 3-5μm in diameter, are located just beneath a thin layer of squamous epithelium which lines the ovigorous folds. Small oocytes about 20-40μm in diameter are found in the vicinity of the germ cells and small chromatin nucleolus stage oocytes.

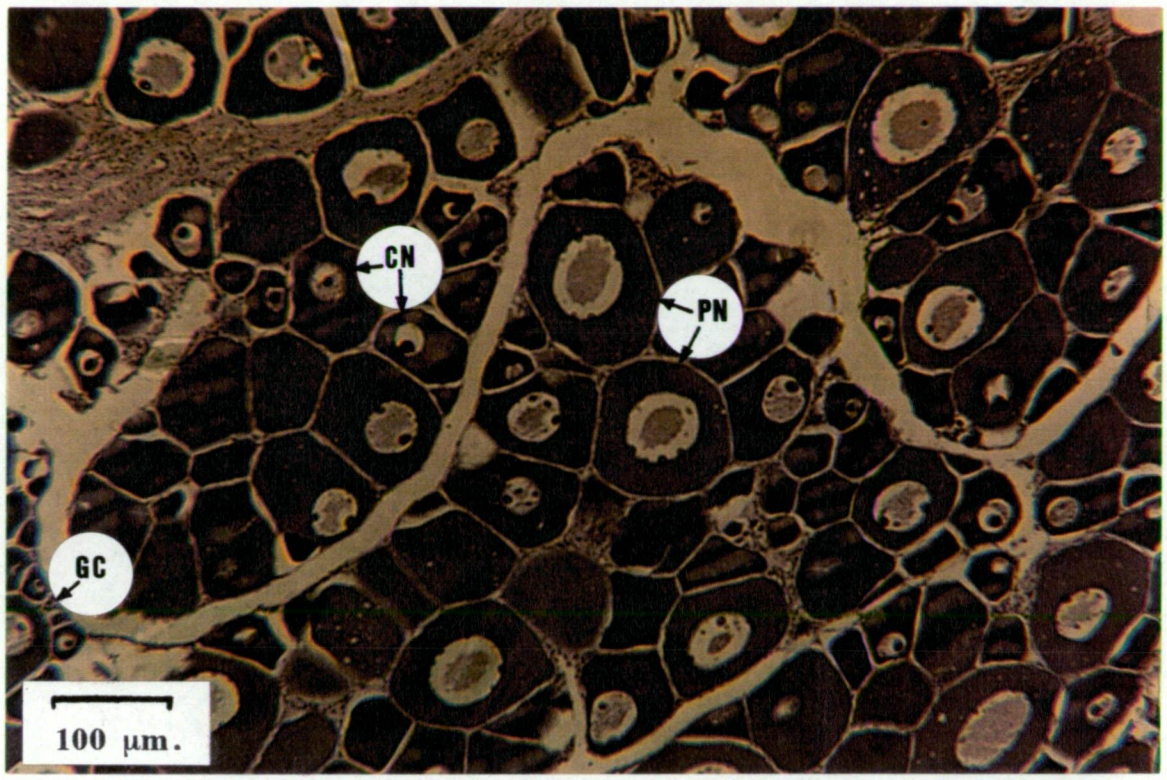


Figure 4.4a Ovigerous lamellae in *Latris lineata* sampled 25 February 1992 (X 100; F.L.= 66cm; G.I.=10.19; Gonad wt.=29.3g) GC = germinal cell; CN = chromatin nucleolus stage oocyte; PN = peri-nucleolus stage oocyte.

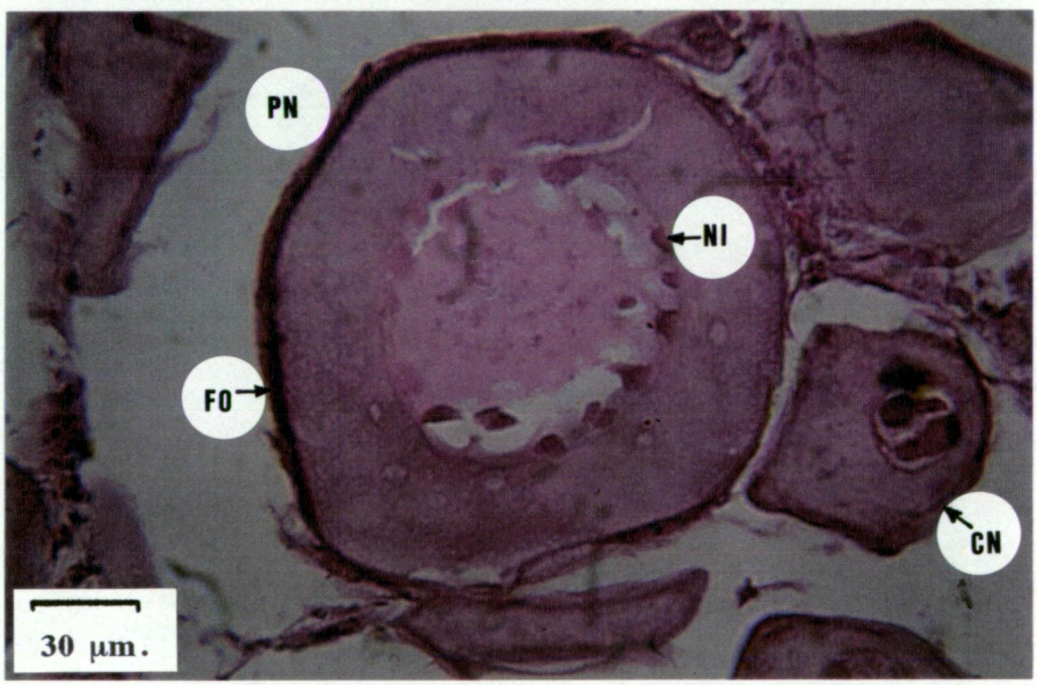


Figure 4.4b Chromatin nucleolus and peri-nucleolus stage oocytes of *Latris lineata* (X 200; F.L.=76.0 cm; G.I.= 16.96; Gonad wt.= 74.5g; 14 June 1991) CN = chromatin nucleolus stage oocyte; PN = peri-nucleolus stage oocyte; FO = follicle; NI = nucleoli.

Various stages of peri-nucleolus type oocytes (Figures 4.4a and 4.4b) are seen in sections of *Latris lineata* ovaries. Peri-nucleolus stage oocytes of *Latris lineata* range between 100μm and 180μm in diameter. These oocytes are of variable shape, although they are generally more rounded than chromatin nucleolus stage oocytes and are characterised by having a relatively greater amount of cytoplasm. The central nucleus contains multiple peripheral nucleoli about 10μm in diameter. A small number of vesicles may occur in the cytoplasm of larger peri-nucleolus stage oocytes however no circum-nuclear ring arrangement of these vesicles is obvious in these oocytes for *Latris lineata*. The developing follicle can be seen surrounding the oocytes at this stage.

4.3.1.2 Cortical alveoli stage.

The cortical alveoli stage of oocyte growth is characterised by the accumulation of clear vesicles (10-15μm in diameter) in the periphery of the cytoplasm as can be seen in Figures 4.5a and 4.5b. Oocytes become markedly larger, increasing in diameter from about 180μm to 350μm during this stage of development in *Latris lineata*. Following the initial appearance of peripheral cortical alveoli, larger empty vacuoles (15-25μm in diameter) can be seen to form as an irregular ring in the vicinity of the centrally-located nucleus and remain isolated from the peripheral cortical alveoli. These vacuoles are oil droplets which latter coalesce to form the oil droplet found in the mature egg. Both the peripheral cortical alveoli and the perinuclear oil droplets appear clear as their contents are lost during conventional procedures followed to prepare and stain samples with eosin and heamatoxylin (Selman and Wallace, 1989; West, 1990). The follicle is distinct at this stage and can often be seen to have separated from the oocyte during histological procedures, to reveal the chorion which has started to form. The presence of cortical alveoli indicates that the female will spawn in the coming season (Bromage and Cumaranatunga, 1988; Morrison, 1990) as this and subsequent stages of oocyte development are gonadotrophin dependant (Wallace and Selman, 1981; De Vlaming, 1983).

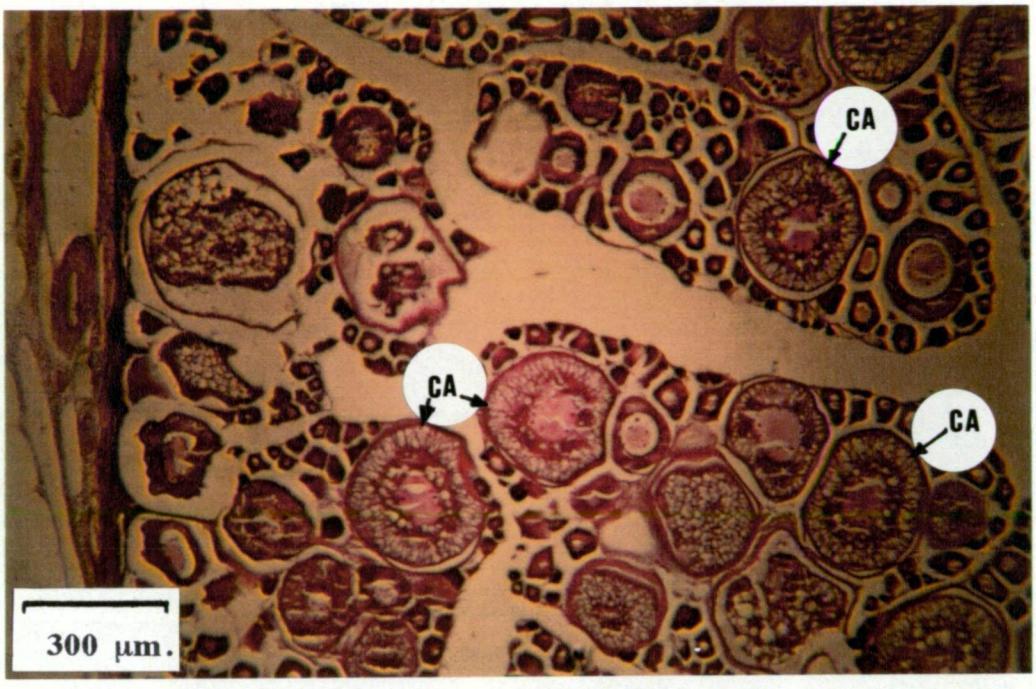


Figure 4.5a Cross section of cortical alveoli stage ovary from *Latris lineata*. (12 July 1990; X 80; F.L.= 60.0cm; G.I. = 14.77; Gonad wt. = 31.9g). CA = Cortical alveoli stage oocyte.

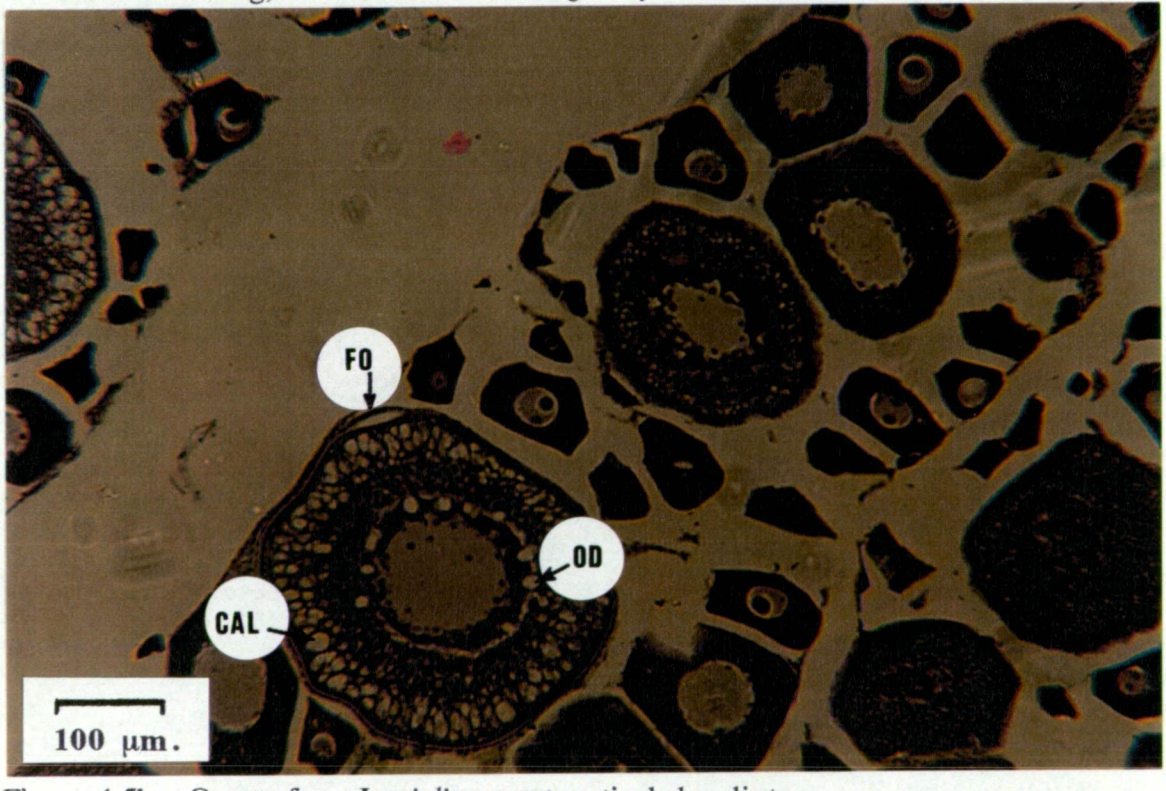


Figure 4.5b Oocyte from *Latris lineata* at cortical alveoli stage. (12 July 1990; X 100; F.L. = 65.0; G.I. = 13.18 Gonad wt. = 36.2g)

CAL = cortical alveoli; OD = oil droplet; FO = follicle.

4.3.1.3 Vitellogenic oocytes.

The commencement of vitellogenesis in *Latris lineata* is marked by the appearance within the cytoplasm of eosinophilic yolk globules, between the chorion and the cortical alveoli, at the periphery of the oocytes (Figure 4.6a). As yolk globules accumulate, oocytes grow from a diameter of 350μm to a diameter of 600μm. The cortical alveoli are initially seen together with the yolk globules in *Latris lineata*, however they are not visible in sections in which yolk globule accumulation is more advanced. Oil droplets are still present as large (20-40μm) clear vacuoles in the perinuclear cytoplasm (Figure 4.6b). The follicle thickens to approximately 12μm and yolk globules 5-15μm in diameter increase in number, occupying a progressively larger proportion of the cytoplasm as the oocyte grows, displacing the oil droplets into a more compact formation around the centrally-located nucleus.

4.3.1.4 Final oocyte maturation.

Following the completion of vitellogenesis the germinal vesicle (nucleus) migrates towards the animal pole of the oocyte. Germinal vesicle migration can be seen in oocytes of *Latris lineata* with diameters between 600μm and 650μm (Figure 4.7a). As this process is occurring, yolk globules and oil droplets may begin to coalesce to form larger bodies in the cytoplasm. Subsequent to this migration, the nuclear envelope dissociates at the periphery of the oocyte and the yolk globules and oil droplets further coalesce (Figure 4.7b) leaving a relatively homogeneous mass of fluid yolk. During this process the oocytes of *Latris lineata* expand to a diameter approaching 900μm before ovulation and further expansion concomitant with hydration in the ovarian lumen.

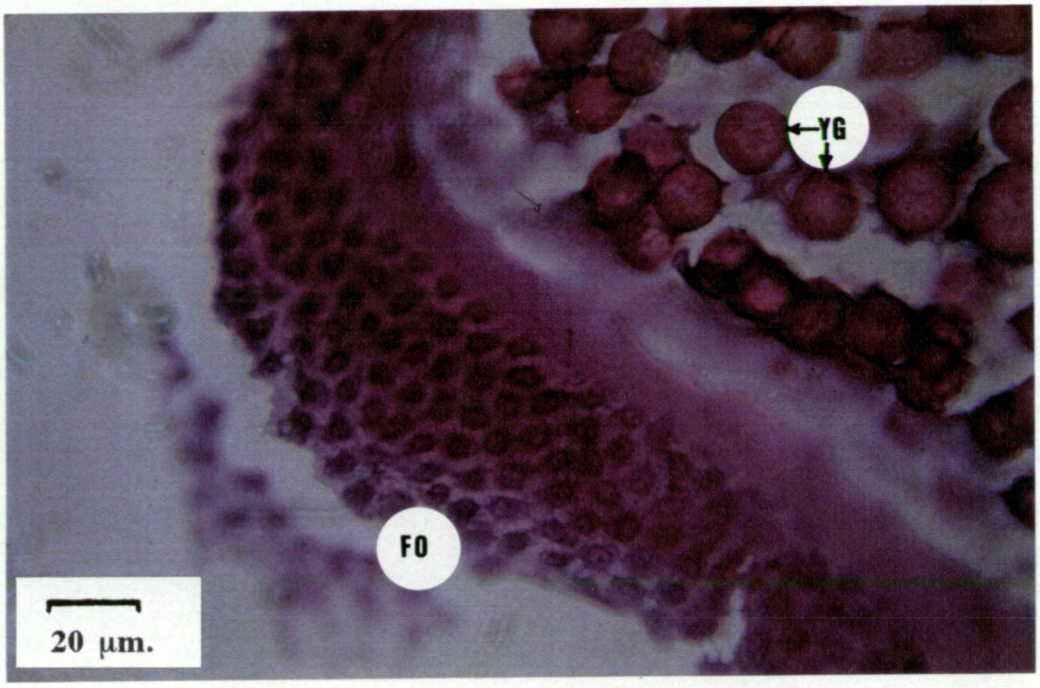


Figure 4.6a Follicle surface of a vitellogenic oocyte in *Latris lineata*.
(X800; 20 Sept.1990; F.L.= 54cm; G.I.= 28.58 Gonad wt.= 45.0g)
FO = follicle (theca); YG = yolk globule.

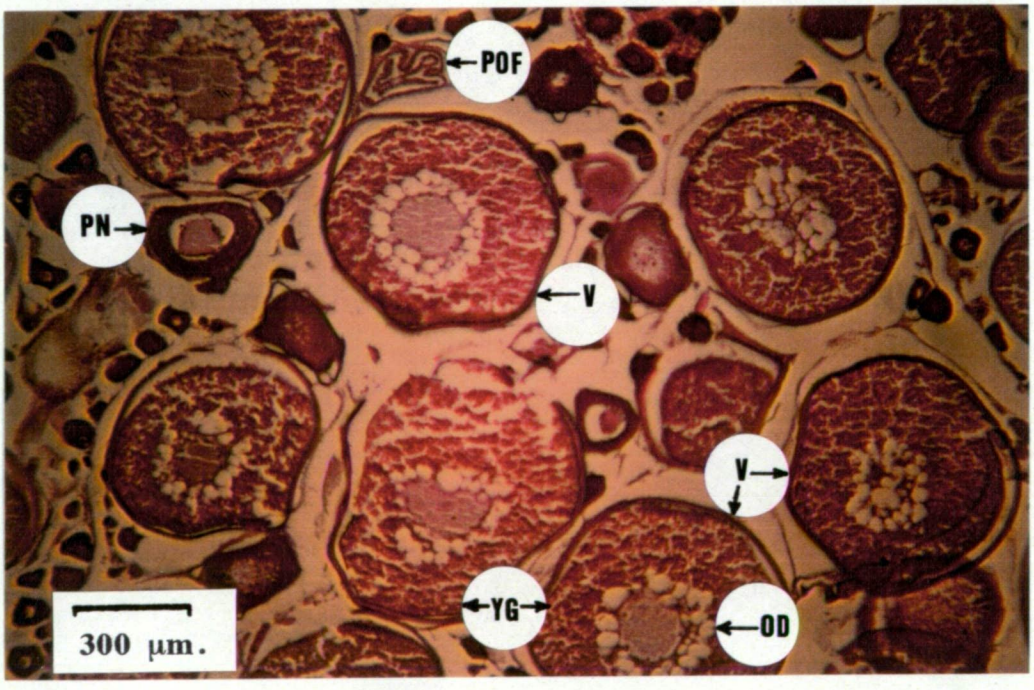


Figure 4.6b Vitellogenic oocytes in *Latris lineata*.
(X200; 20 Sept.1990; F.L.= 56.5cm; G.I.= 34.93; Gonad wt.= 63.0g)
PN = Peri-nucleolus stage oocyte; V = vitellogenic oocyte; OD = oil droplet;
YG = accumulated yolk globules; POF = post-ovulatory follicle

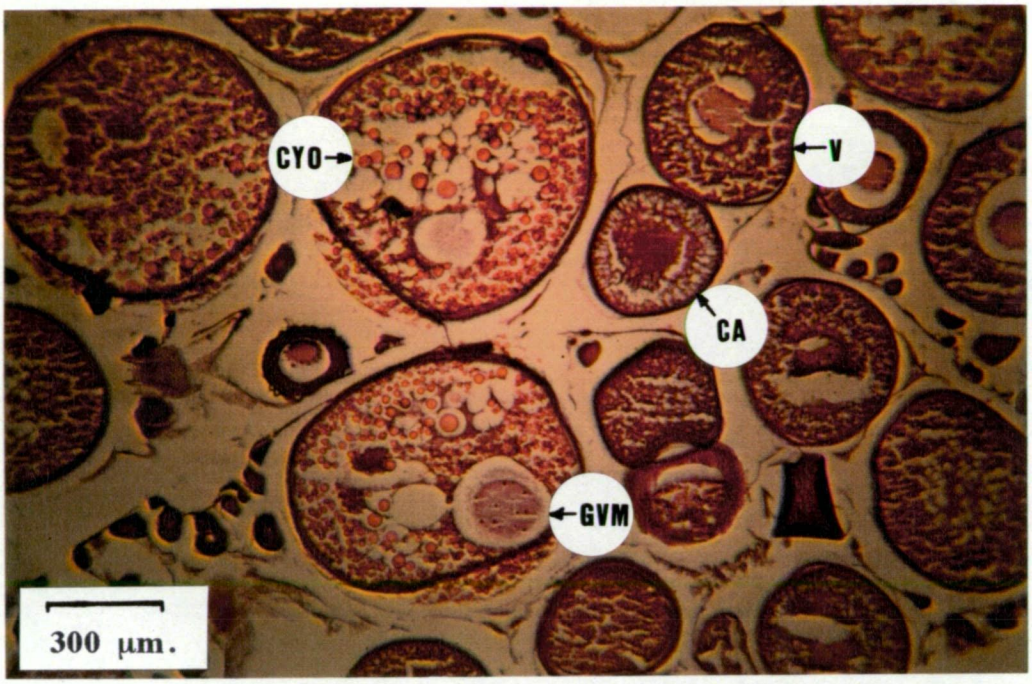


Figure 4.7a Oocytes from *Latris lineata* at germinal vesicle migration (GVM) stage of development, also showing other oocytes at various stages of development. (X80; 29 Sept.1990; F.L.= 78.5cm; G.I.= 91.64; Gonad wt.= 443.3g) V = vitellogenic oocyte; GVM = oocyte ar germinal vesicle migration stage; CA = oocyte at cortical alveoli stage; CYO = yolk globules and oil droplets

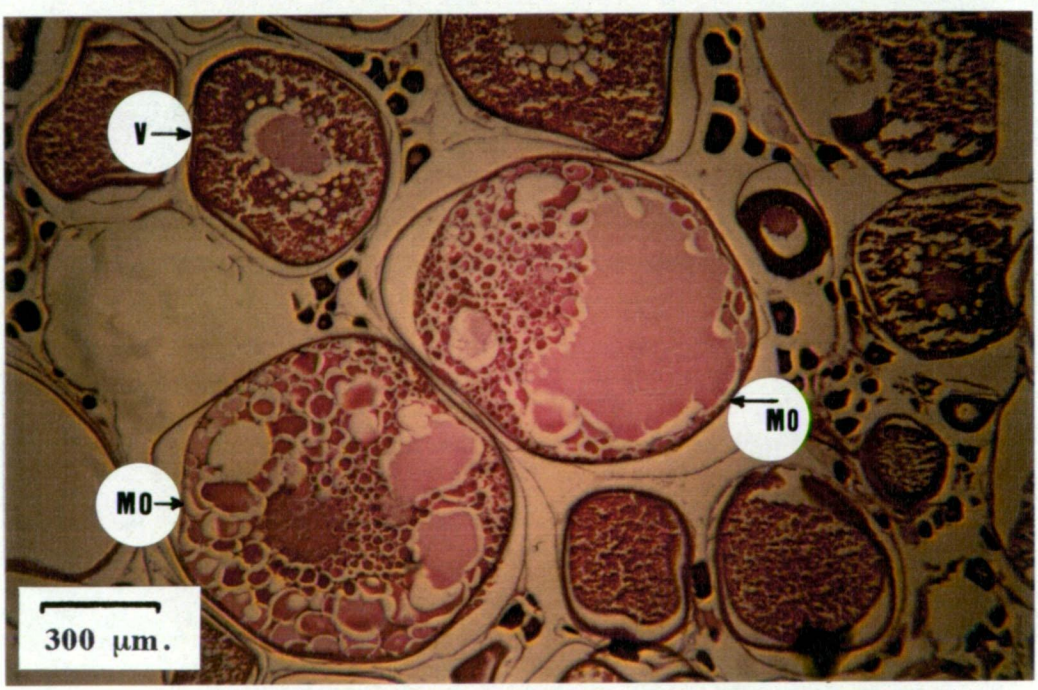


Figure 4.7b Oocyte of *Latris lineata* at final maturation stage of development. (X80; 29 Sept.1990; F.L.= 52.0cm; G.I.= 55.05; Gonad wt.= 77.4g) MO = mature oocyte; V = vitellogenic oocyte.

4.3.2 Classification of stage of reproductive development in female *Latris lineata*, based on histological assessment.

After extensive examination of histological samples during oocyte measuring procedures, the following classification system was devised to represent best the stages of reproductive development in female *Latris lineata*.

Classification	Description
Primary oocytes only (PN)	Only chromatin nucleus and peri-nucleolus stage oocytes present. Such samples could be from developing virgins or recovering mature fish.
Cortical alveoli stage (CA)	Cortical alveoli stage oocytes present together with primary stage oocytes.
Vitellogenic (VC)	A range of sizes of vitellogenic oocytes are present, showing varying amounts of accumulated yolk visible as eosinophilic globules in the cytoplasm.
Germinal vesicle migration (GVM)	Vitellogenesis complete and germinal vesicle no longer centrally located. Yolk globules and oil droplets may have commenced coalescing.
Mature oocytes (MAT).	Germinal vesicle no longer present (GVBD complete) with yolk coalescence progressed to varying degrees leaving an abstract formation of fluid yolk (not confined in globules) throughout the oocyte.
Spent fish (NT).	Many post-ovulatory follicles can be seen in various states of resorption in a generally more open arrangement of oocytes. Degenerating vitellogenic stage oocytes may be present, or primary growth stage oocytes only. No Cortical alveoli stage oocytes present.

4.3.3 Histological description of reproductive development in male *Latris lineata*.

The testes of *Latris lineata* are of the lobule type described by Billard (1982) and Nagahama *et al.* (1982) with tubules arranged in an anastomosing network as portrayed by Grier (1981). The following classifications were constructed to describe best the stages of testis development observed in *Latris lineata* after consulting other classification systems (Davis, 1982; Pankhurst *et al.*, 1987; Young *et al.*, 1987) themselves based on histological assessment.

4.3.3.1 Immature (I) and developing virgins (DV).

Testes of both immature and developing virgin male *Latris lineata*, are small in cross section and display a cellular structure composed of interstitial cells, which appear vacuolated, together with spermatogonia. Lobule lumina and duct networks are poorly developed but are visible. Lobules are generally compact which gives the testis cross section a mesh-like appearance which becomes marginally more open towards the interior where ducts predominate. Differentiation between immature and virgin males is somewhat subjective but is based on assessing the cross-sectional area of the sample, with immature testes being markedly smaller than those of developing virgin males. The testes of developing virgins often have a darker staining band, which may represent a growth margin, following the peripheral margin (Figure 4.8) of the organ.

4.3.3.2 Spent /recovering(SR).

No immediate post spawning, "spent" male *Latris lineata* were sampled in this study. The nearest post spawning fish were sampled on 22 January 1992 and were categorised as being "spent recovering". Spent recovering testes vary from those of immature and developing virgin males, generally being distinctly larger in cross sectional area and having a more open channel network. A thin zone of spermatogonia lines the well defined walls of the lobules and remnant, degenerating spermatozoa can often be seen in the wider internal ducts of spent recovering testis.

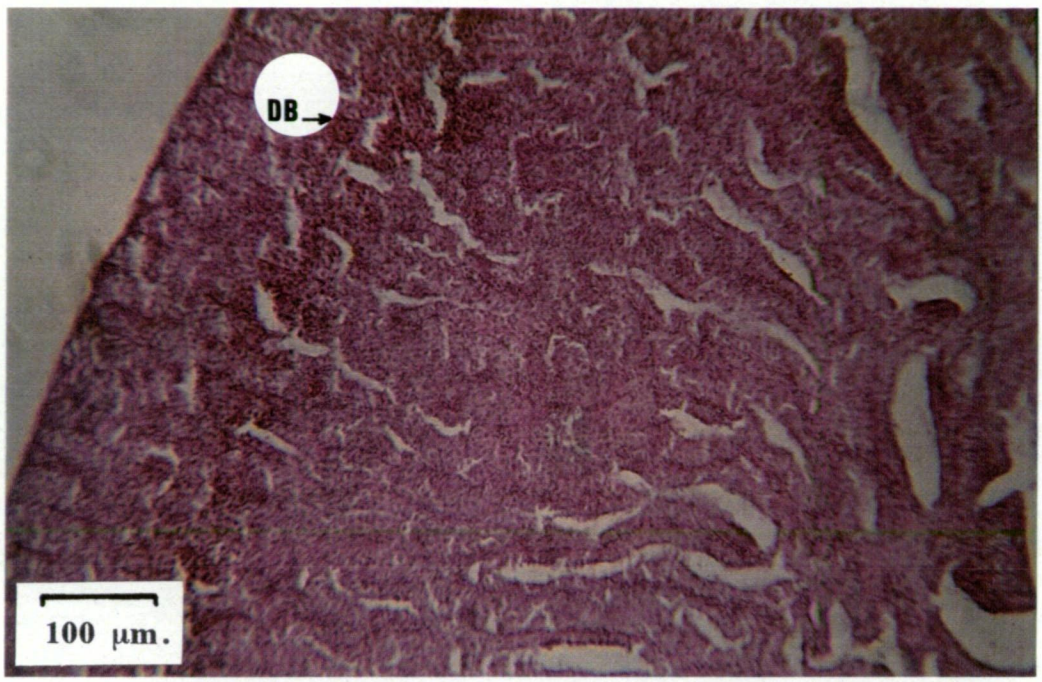


Figure 4.8 Cross section of testis from developing virgin male *Latris lineata*.
(X200; 14 March 1991; F.L.=55.0cm; G.I.= 1.32; Gonad wt.= 2.2g).
DB = dark staining band.

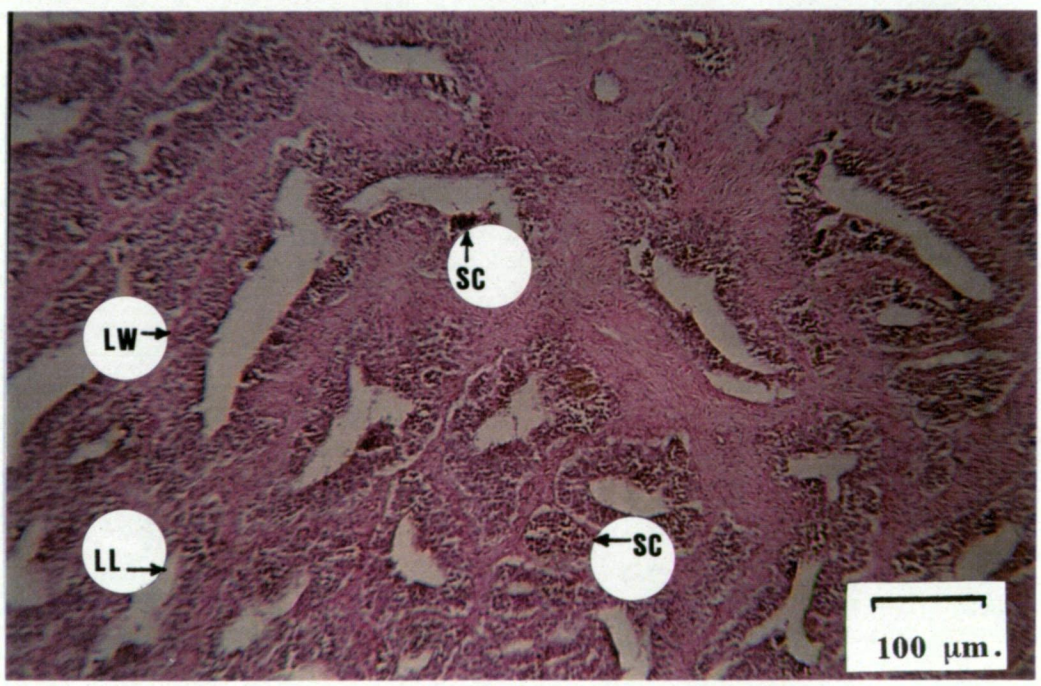


Figure 4.9 Cross section of developing (M1) testis of *Latris lineata*.
(X200; 14 June 1991; F.L.= 65.0cm; G.I.= 6.01; Gonad wt.=16.5g)
SC = Spermatocytes; LL = lobule lumina; LW = lobule wall.

4.3.3.3 Developing (M1).

With the onset of spermatogenesis nests of spermatogonia and primary and secondary spermatocytes can be identified along the lobules and the proportion of interstitial cells appears reduced (Figure 4.9). The spermatogenic cells are still dispersed along the lobule lumina and no spermatids are present.

4.3.3.4 Maturing (M2).

With further maturation there is a proliferation of spermatocytes lining the lobules which have widened. Nests of spermatids have developed, but these are not dominant and no spermatozoa are present in lobule lumina or ducts. Maturing testes still retain an open mesh-like appearance in cross section.

4.3.3.5 Mature (M).

In a mature testis spermatozoa can be found in the lobule lumina and sperm ducts although they do not fill these structures (Figure 4.10). Nests of spermatids are common and many can be seen to be undergoing spermiogenesis. Spermatozoa are arranged radially with their heads orientated outwards and flagella trailing to the centre of the cyst, prior to rupture. Interstitial cells are not obvious at this stage as following extensive proliferation, spermatogenic cells now dominate.

4.3.3.6 Running-ripe (R).

The lobule lumina and sperm ducts are packed with spermatozoa. Spermatozoa also fill the vas deferens where they accumulate (Figure 4.11), as there is no specific sperm storage structure in teleosts (Billard *et al*, 1982). At this stage the lobules have expanded laterally and longitudinally to occupy most of the testis and intralobular tissue is restricted to the formation of thin lobule walls. Cysts of spermatids and spermatocytes are still present at irregular intervals along the lobule lumina of ripe testes.

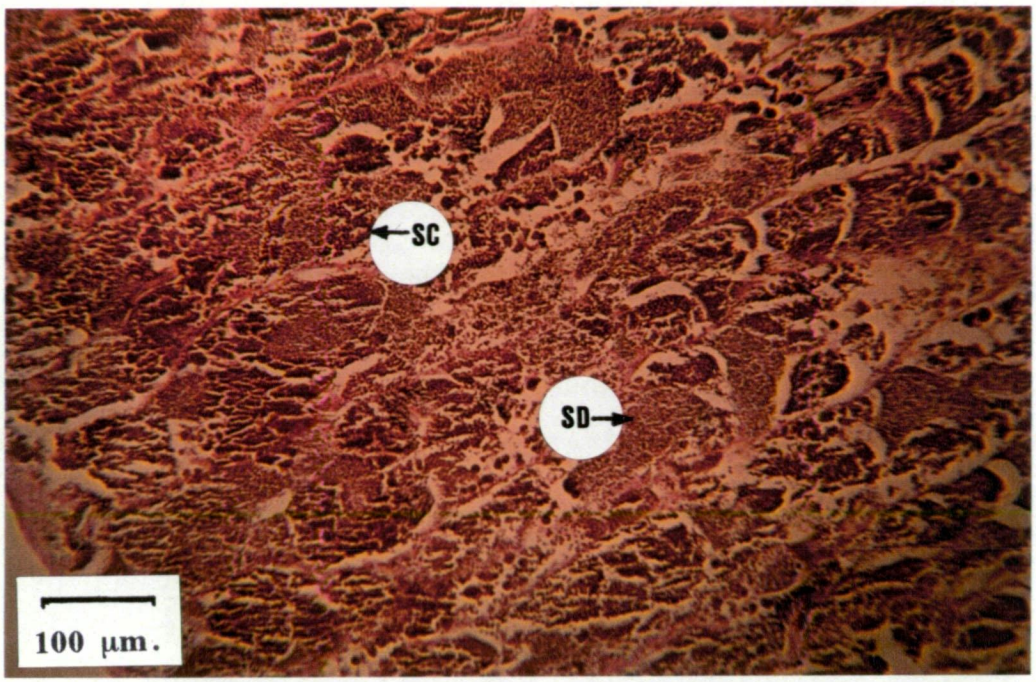


Figure 4.10 Cross section of mature (M) testis from *Latris lineata*. (X200; 12 July 1990; F.L.= 58.5cm; G.I.= 51.90; Gonad wt. = 103.9g) SC = spermatocytes; SD = spermatids.

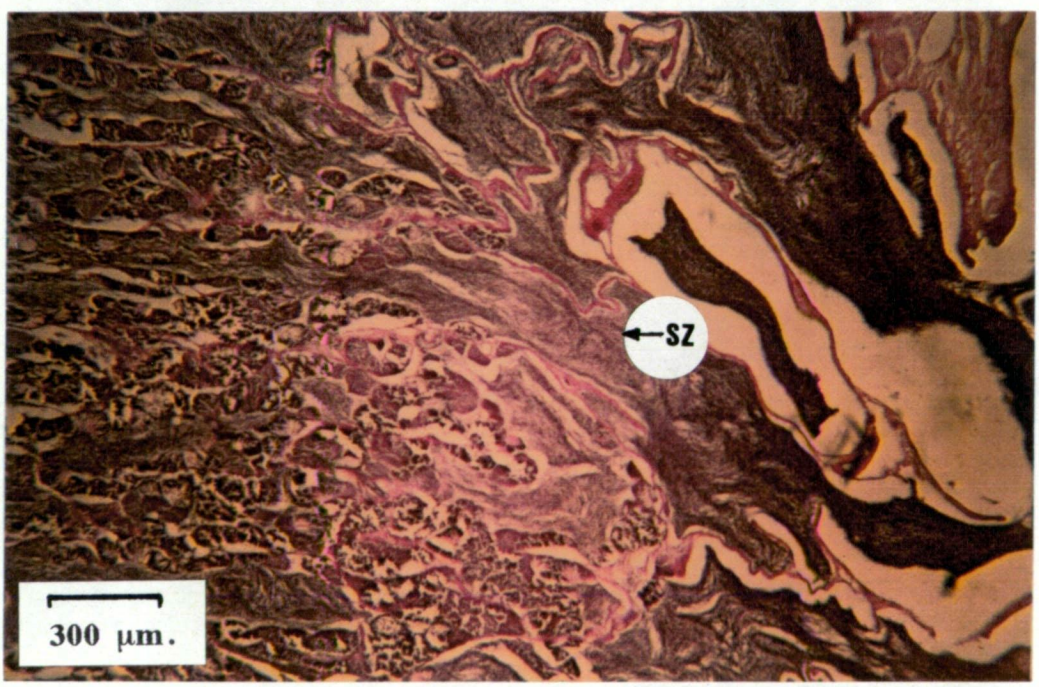


Figure 4.11 Cross section of running- ripe (R) testis from *Latris lineata*. (X80; 29 Sept.1990; F.L.= 59.0cm; G.I.= 65.15; Gonad wt. = 133.8g) SZ = spermatozoa.

4.4 Macroscopic description of gonadal development in *Latris lineata*.

Although histological methods are precise, other methods for reporting the stage of reproductive development are available which are simpler, more immediate and require less complex resources to obtain the information desired. Staging of gonads based on their external 'macroscopic' appearance is the easiest technique, however it is recognised as being a subjective measure which should be validated by other measures of reproductive development (West,1990) such as a full histological description.

4.4.1 Macroscopic description of testis development.

The testes of *Latris lineata* are located in the same position as the ovaries (Figure 4.5). The testes are suspended from the wall of the body cavity, beneath the swim bladder by peritoneal membranes known as 'mesorchia' (Abu-Hakima, 1984; Morrison, 1990). Through this membrane runs the major blood vessel which separates at the point of connection to the testes into two vessels. Each of these vessels supplies one organ, running in a longitudinal furrow (Figure 4.14). The shape of testes vary from being roughly triangular, to kidney shaped in cross section with one side invaginated to form a centrally located area from which blood vessels and sperm collecting ducts radiate to the lobes to either side.

4.4.1.1 Immature.

The testes from a small number of immature males (n=6) were collected in this study. Although histologically similar to virgin fish, the testes of immature males are very small, appearing thin and thread-like, with a weight of less than 1.0g (Figure 4.12).

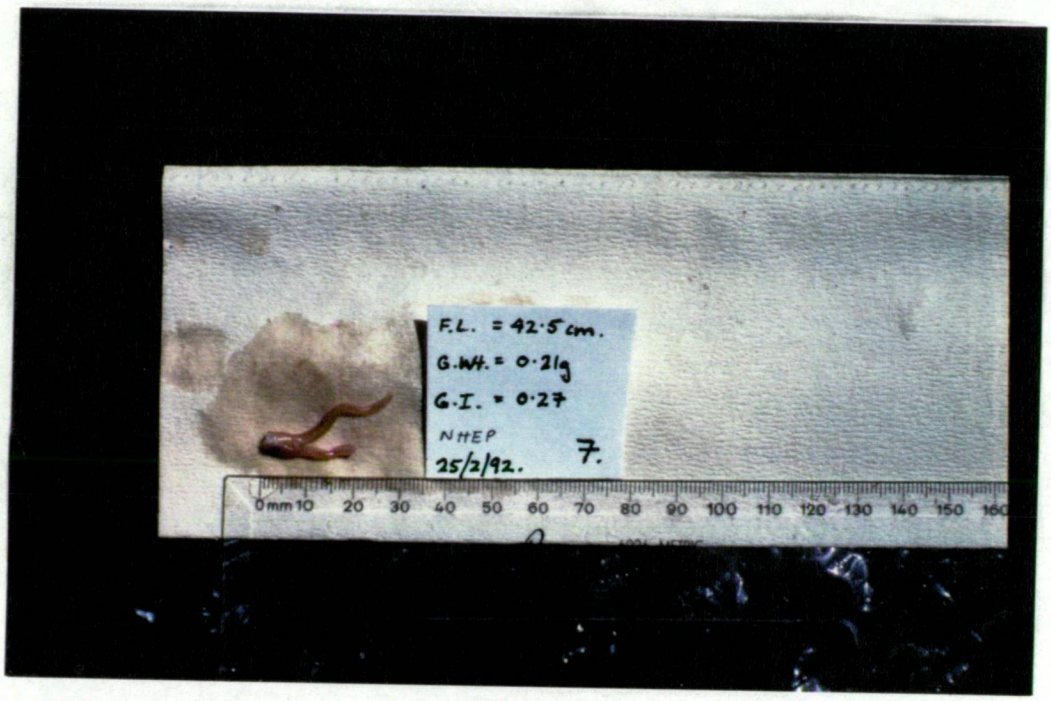


Figure 4.12 Testis from immature (I) male *Latris lineata*.

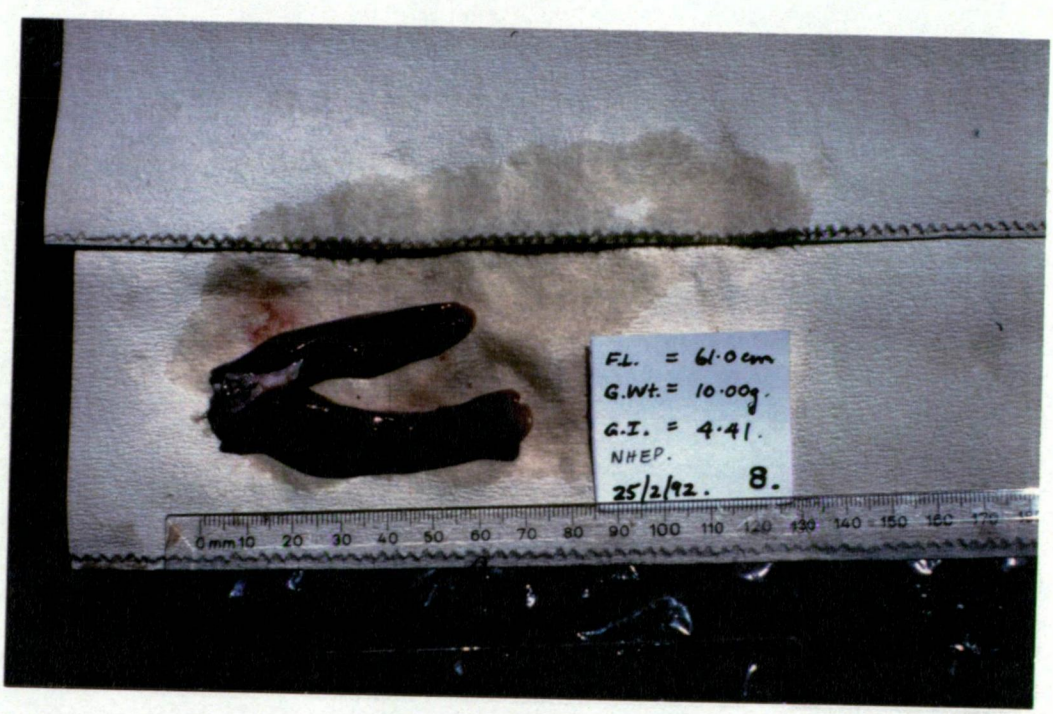


Figure 4.13 Testis from spent recovering (SR) male *Latris lineata*.

4.4.1.2 Developing virgin.

Testes of developing virgin males are small but are sufficiently developed to be readily recognisable as testes. These organs have filled out to some extent, generally appearing elongate and cream coloured.

4.4.1.3 Spent recovering.

Spent recovering testes generally appear creamy-brown but may be dark brown in colour (Figure 4.13). Longitudinal furrows are deep and the cross sectional area of each organ is generally greater than that of developing virgins. A number of striations are often present on the external surface.

4.4.1.4 Maturing/mature.

As testes undergo spermatogenic development they increase in size and gradually become more white in colour. During maturation the cross sectional area of the testis increases and the longitudinal furrow which accommodates the sperm collecting duct and blood vessels (Figure 4.14), becomes enveloped by the expanding lobes on either side. Milt will not flow freely from the cut surface of the testis at early stages of maturation (maturing). However, viscous milt will flow from the cut surface and small amounts can be expressed from the sperm duct when the testis is at the mature stage of development.

4.4.1.5 Running-ripe.

Running-ripe testes are large, white and have blood vessel obvious on the surface. Copious amounts of milt will flow freely from the cut surface and from the sperm duct (Figure 4.15).

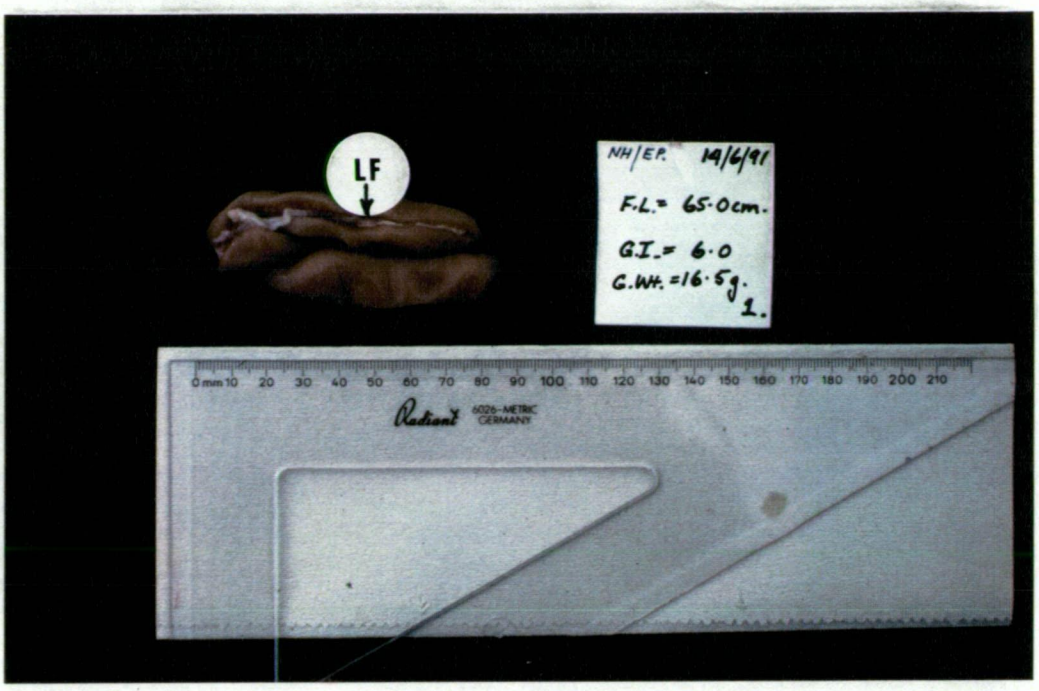


Figure 4.14 Testis from recovering maturing (M1) male *Latris lineata*.
LF = Longitudinal furrow.

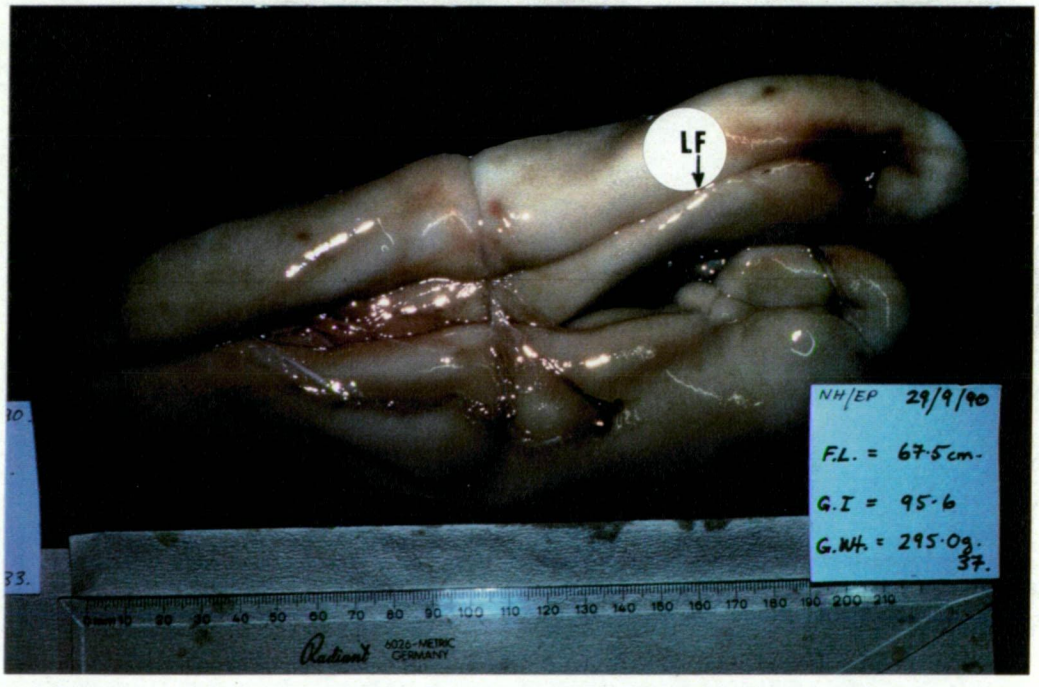


Figure 4.15 Testis from recovering running-ripe (R) male *Latris lineata*.
Longitudinal furrows enveloped by lobes of each organ.
LF = longitudinal furrow.

4.4.2 Macroscopic description of ovarian development.

The position within the body cavity occupied by the ovaries of *Latrislineata* is shown in Figure 4.5. The ovaries are paired structures suspended underneath the swimbladder, by peritoneal membranes, referred to as 'mesovaria' (Morrison, 1990), extending from the dorsal lining of the body cavity. Both ovaries share a common oviduct which opens at the genital pore posterior to the rectum and anterior to the urinary pore in the urogenital papilla. The ovary is supplied with nerves and blood vessels which communicate to the organ within the mesovaria.

4.4.2.1 Developing virgins.

No ovaries sampled in this study could be readily identified as being from immature female fish. Many fish sampled were classified as being 'developing virgin' females. These fish had developing ovaries which were small and cylindrical. The walls of these ovaries were thin with a translucent pink coloured matrix within which no oocytes were visible to the naked eye (Figure 4.16).

4.4.2.2 Spent recovering.

Ovaries of fish recovering from the previous spawning season are larger than ovaries of developing virgins. These ovaries have a thicker wall and vary from orange-brown in colour and flabby, in medium size fish (Figure 4.17), to dark reddish-brown and very flaccid in large fish. Many brown bodies, representing remnant atretic oocytes, were easily visible within the ovarian tissue of spent recovering females.

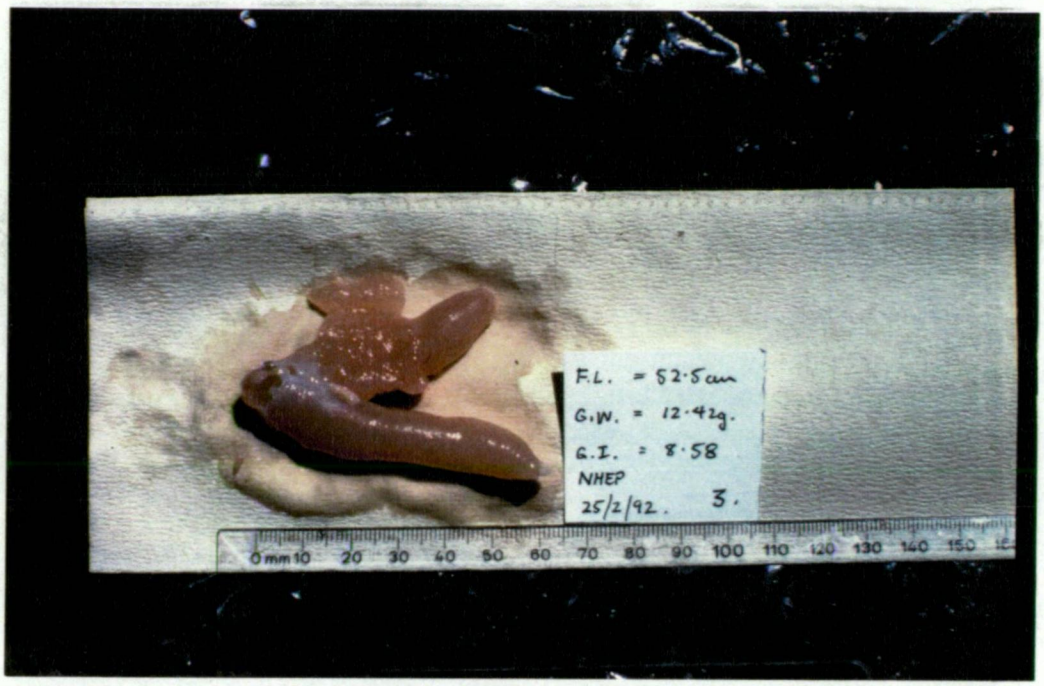


Figure 4.16 Ovaries from developing virgin (DV) female *Latris lineata*.

Figure 4.17 Spent recovering (SR) ovaries from mature female *Latris lineata*.

4.4.2.3 Developing.

With the commencement of cortical alveoli formation, the ovaries become firmer and barely visible oocytes can be distinguished in the translucent ovarian matrix; although these are difficult to observe through the wall of the ovary.

4.4.2.4 Maturing/mature.

In this study no samples were obtained of ovaries at intermediate stages of vitellogenesis. Ovaries from fish captured during the spawning season contained oocytes which had completed vitellogenesis and were entering the early stages of final oocyte maturation. These large ovaries varied in colour from golden-yellow (Figure 4.18) to creamy-yellow (Figure 4.19) and were uniformly composed of opaque oocyteswhich were readily visible through the wall of the ovary.

4.4.2.5 Running ripe.

The ovaries of running ripe females are differentiated from those of maturing fish by the presence of hyaline oocytes in the ovarian matrix which were easily visible through the wall of the ovary. A free-flowing mass of hydrated eggs could be expressed from the vent with light pressure on the abdominal wall of running ripe females.

4.4.2.6 Spent.

Immediate post-spawning "spent" ovaries were rounded and appeared similar to spent recovering ovaries. However, the ovarian wall was thicker, the organ was firmer and remnant degenerating oocytes were often present.

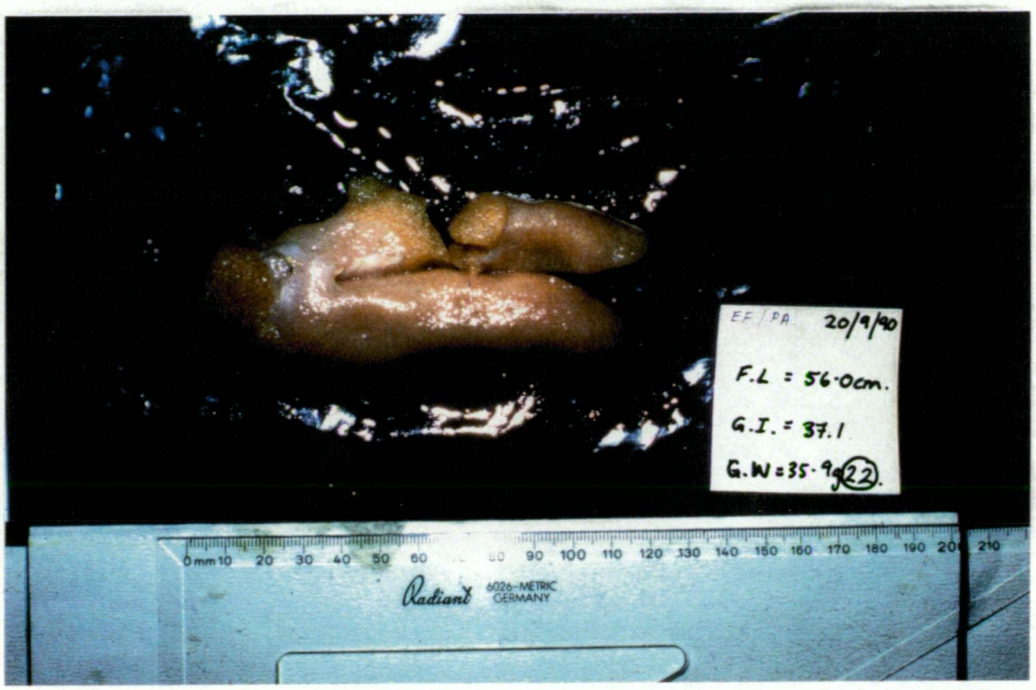


Figure 4.18 Mature ovaries from *Latris lineata* with oocytes at germinal vesicle migration (GVM) stage of development. (Note G.I. = 37.1)

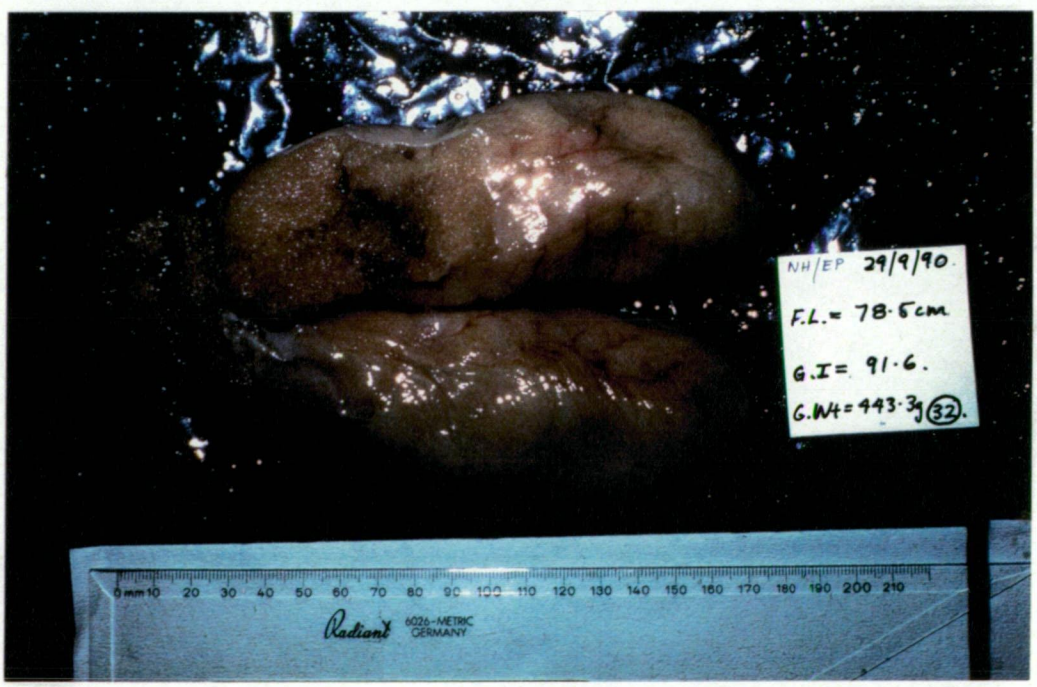


Figure 4.19 Mature ovaries from *Latris lineata* with oocytes at germinal vesicle migration stage (GVM) of development. (Note G.I. = 91.6).

4.5 Description of the annual pattern of reproduction in *Latris lineata*.

A number of methods used to descibe reproductive development in females, introduced in the review by West (1990) are applicable to male fish also. A gonad index is commonly used to describe the pronounced annual changes in the relationship between gonad weight and a measurement of body size (weight or length). De Vlaming *et al.* (1982) investigated the assumption that the effects of differential body sizes among sampled animals could be "normalised" by expressing gonadal weight as a percentage of body weight (100 x gonad weight/body weight). Using data from the fluffy sculpin, *Oligocottus snyderi* and inland silversides, *Menidia beryllina*, the authors concluded that the ovarian weight to body weight ratio is not always the best way of expressing the gonad index. These authors state that for *Oligocottus snyderi* the correlation coefficients between gonad weight and standard length or the cube of standard length, are higher than those for ovarian weight and body weight. It is suggested that expressing ovarian weight as an exponential function of body size rather than body weight, may provide a more accurate gonad index (De Vlaming *et al.*, 1982).

As stated in section 2.1, accurate weighing of fish was not always possible under the sampling conditions prevailing in this study. The gonad index selected for use in this study is the common index used by Cayre and Laloe (1986), which expresses gonad weight (g) as an exponential function of fish length (cm).

$$\text{Gonad index} = \frac{\text{Gonad weight}}{\text{Fork Length}^3} \times 10^5$$

The gonad index values recorded for all fish sampled between 25 May 1990 and 22 May 1992 are presented in Figure 4.20a (female fish) and 4.20b (male fish). These figures show that in Tasmanian waters the annual spawning season of *Latris lineata*

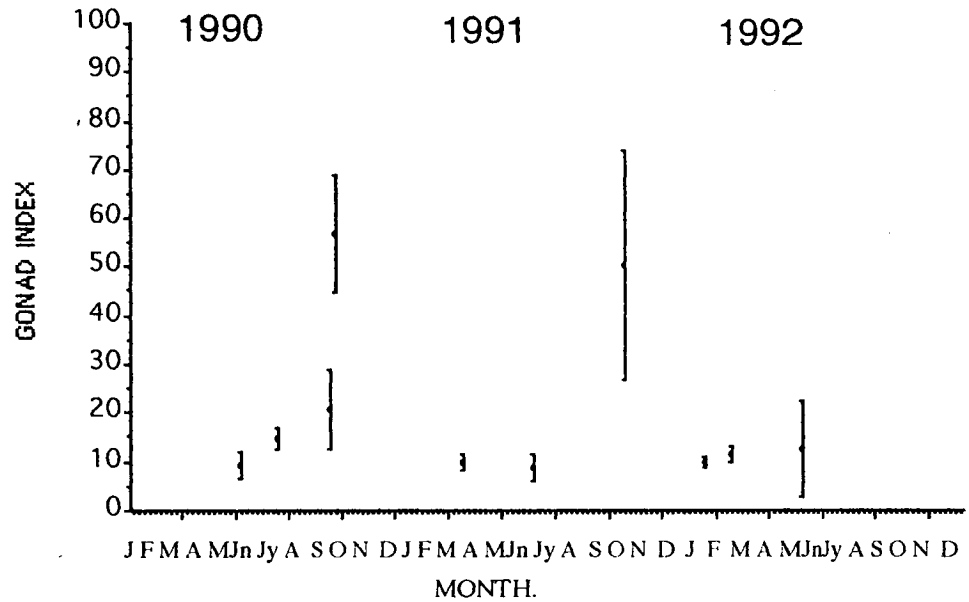


Figure 4.20a Mean and 95% confidence intervals for gonad index values of all female *Latris lineata* sampled between 25 May 1990 and 22 May 1992 (N=175).

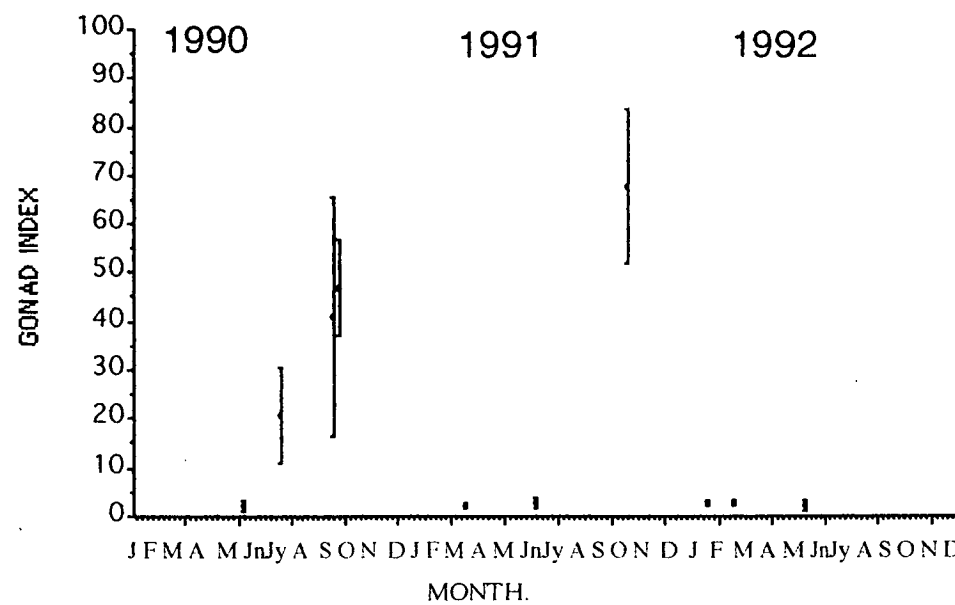


Figure 4.20b Mean and 95% confidence intervals for gonad index values of all male *Latris lineata* sampled between 25 May 1990 and 22 May 1992 (N=159).

extends from September into October, although the length of the spawning season is not clearly identified. Personal observations of commercial fishermen suggest that *Latris lineata* have completed spawning by November.

Two way ANOVA comparing sampling date and sex with gonad index, assuming homogeneity of variance, shows that there is no significant difference ($P > 0.05$) between the mean gonad indices of male and female fish (Appendix 7). Sampling date has a significant ($P< 0.05$) effect on mean gonad index as would be anticipated. The interaction between sex and sampling date also has a significant ($P <0.05$) effect on mean gonad index, suggesting that the differences between the mean gonad index of male and female fish vary with the sampling month.

It is accepted that no one year is adequately represented by these data. However, combination of all data arranged in monthly chronological order (Table 4.1), allows a cautious interpretation to be conducted. Two sample t-tests for each sampling month reveal that the gonad indices of male fish is significantly lower ($P< 0.05$) than that of female fish during the period from January through to June (Table 4.1). Data collected on 12 July 1990 suggests that gonad development is initiated at about this time as gonad index of both sexes is no longer significantly different ($P> 0.05$). One way ANOVA's comparing gonad index of male fish at the different sampling months (Appendix 9A) and gonad indices of female fish (Appendix 9B) at the different sampling months, suggest that male fish commence gonad development in advance of females. There is a significant difference ($P< 0.05$) in the mean gonad index of male *Latris lineata* sampled in July (G.I. = 20.54 +/- 14.96) compared to the mean gonad indices of samples representing the previous months (mean G.I. = 2.55 +/- 0.26; n=6), while the gonad index of female fish fish sampled at this time (G.I. = 14.03 +/- 2.73) were not significantly different ($P >0.05$) from those recorded for these previous months (mean G.I = 10.38 +/- 1.53, N=6). These conclusions must be

treated circumspectly, given limitation that that the data in question has been compiled from 3 sampling years.

Table 4. 1 Mean gonad index values for male and female *Latris lineata*. (Data arranged in monthly chronological order ignoring years, N=334).

SAMPLING DATE		MALE FISH Mean gonad index		+/- S.D.	N	FEMALE FISH Mean gonad index		+/- S.D.	N
22 Jan.'92	*	2.98	a	1.56	(31)	9.83	a	3.07	(44)
25 Feb.'92	*	2.55	a	1.25	(20)	11.71	a	3.48	(20)
14 Mar.'91	*	2.41	a	1.47	(25)	10.22	a	3.91	(27)
22 May '92	*	2.26	a	1.56	(8)	12.69	a	4.03	(3)
25 May '90	*	2.41	a	1.38	(10)	9.02	a	4.05	(10)
14 Jun.'91	*	2.70	a	1.76	(9)	8.33	a	4.35	(12)
12 Jul.'90		20.54		14.96	(13)	14.03	ab	2.73	(12)
20 Sep.'90	*	41.25	b	26.67	(7)	20.88	b	16.59	(18)
29 Sep.'90		47.06	b	25.87	(28)	56.83	c	22.86	(17)
4 Oct.'91		59.21	b	28.74	(8)	46.23	c	36.21	(12)

* = male and female mean G.I is significantly different ($P < 0.05$) for sampling month. Sampling dates sharing similar superscripts within each column show no significant difference ($P > 0.05$) in mean G.I.

The information gained from gonad index data provides a description of the annual pattern of gonad development. However, it is not independent of fish size and cannot accurately predict maturity stages (West, 1990). As only a small number of fish where collected on each sampling occasion (mean no. of fish captured 33.4 +/- 19.5; minimum = 11, maximum = 75) it was not statistically feasible to standardise the size composition of samples to overcome the size dependance as suggested by West

(1990). However, this was fulfilled to some extent by the limited size range of fish captured as 296 (88.6%) of the 334 fish sampled were between 50.5 cm F.L. and 70.0 cm F.L. (Figure 2.6; Appendix 10).

Although it was possible to stage gonads macroscopically during sampling, no such assessment was undertaken as the objective of this component of the study was to describe gonad development from microscopic observation. Classification of the stage of gonad development is based on identifying the most advanced type of oocytes present in female fish (West, 1990). Classification systems for male fish based on histological assessment use a similar approach, by identifying the proliferation of the most advanced spermatogenic cell types. Samples of *Latris lineata* gonads, from which histological sections were prepared (section 4.2), were classified based on the maximum stage of reproductive development present (section 4.3). Oocytes were measured (section 4.2.2.) for female fish (n = 125) and the mean of the 10 largest oocytes recorded was calculated for each sample (Appendix 5). Results for female fish are presented in Table 4.2, while results from male fish are presented in Table 4.3, together with gonad index data for each gonad development classification. One way ANOVA comparing stage of gonad development and mean gonad index is included for male fish (Appendix 11A) and female fish (Appendix 11B) as is one way ANOVA data for stage of gonad development and mean oocyte diameter of female fish (Appendix 12).

These results show that although there was a significant ($P< 0.05$) difference in mean oocyte diameter between primary and cortical alveoli stages of oocyte development stages, there is no corresponding significant ($P> 0.05$) rise in gonad index between these stages. Mean oocyte diameter increases significantly ($P< 0.05$) concomitant with vitellogenesis but does not increase significantly ($P> 0.05$) during germinal vesicle migration. However, a further significant increase ($P< 0.05$) in mean oocyte diameter was shown during final maturation. The mean gonad index of fish sampled

with mature oocytes were not significantly different ($P< 0.05$) from the gonad index of fish at the germinal vesicle migration stage. Pre-spawning fish with mature oocytes would be expected to have a high gonad index. This suggests that these fish had recently spawned and the mature oocytes observed were remnant oocytes. It could be that these fish spawned, or partially spawned, in the holding well prior to sampling, as this has been reported by commercial fishermen and these fish (n=3) had spent one night in captivity.

Table 4.2 Mean oocyte diameter (μm) and gonad index for stages of gonad development in female *Latris lineata* as determined by histology.

STAGE OF DEVELOPMENT	MEAN OOCYTE DIAMETER (μm)	+/- S.D.	N	MEAN GONAD INDEX	+/- S.D.	N
Primary stage oocytes (PN).	127.30	14.77	(72)	10.23 [a]	3.91	(74)
Cortical alveoli stage (CA).	254.50 [a]	45.82	(21)	10.94 [a]	4.54	(22)
Vitellogenic stage (VC).	605.26 [b]	36.64	(5)	42.78 [b]	9.73	(5)
Germinal ves. migration. (GVM)	622.14 [b]	38.04	(18)	53.15 [c]	20.82	(18)
Mature oocytes (MAT).	849.37	30.09	(3)	43.75 [bc]	13.28	(3)
Spent fish (NT)	277.43 [a]	248.87	(3)	14.98 [a]	3.00	(3)

Stages of development sharing similar superscripts within each column show no significant difference ($P > 0.05$)

There was no significantly difference (P> 0.05) in the mean gonad index of immature, developing virgin, spent recovering and developing classifications for male fish (Table 4.3). This suggests that although the early stages of spermatogenesis in *Latris lineata* can be differentiated at the cellular level, these changes are not accompanied by any substantial increase in testis weight. The latter stages of testis development can all be identified by significant differences (P< 0.05) in gonad index.

Table 4.3 Mean gonad index (see text) for stages of gonad development in male *Latris lineata* as determined by histology (N=91).

STAGE OF DEVELOPMENT	MEAN GONAD INDEX	+/- S.D.	N
Immature (I).	0.46 [a]	0.15	(6)
Developing virgin (DV).	1.36 [a]	0.48	(15)
Spent recovering (SR).	3.25 [a]	1.40	(34)
Developing (M1)	3.64 [a]	2.20	(16)
Maturing (M2).	18.72	7.91	(7)
Mature (M).	45.99	10.63	(3)
Running-ripe (R).	63.19	12.64	(10)

Stages of development sharing a common superscript are not significantly different (P>0.05).

Comparison of gonad index with stage of gonad development as determined by histology, for both male and female *Latris lineata*, illustrates the inability of the gonad index to differentiate important gonad development events occurring at the cellular level, an opinion shared by Cayre and Laloe (1986) and West (1990). Problems arise in both sexes when cellular developments during early gametogenesis are not accompanied by corresponding significant increases in gonad size. A similar situation is reported by Hay (1985) for *Clupea harengus pallasi*, who acknowledging that a simple gonad index (gonad weight as a percentage of body weight) may not reveal early gonad development that observed visually in this species. Alternatively problems arise when gonads of female fish have lost weight due to recent spawning but will be replenished before further spawnings. It is proposed that in this situation the gonad index is not able to distinguish accurately between these latter stages of gonad development (following the completion of vitellogenesis), a limitation which is of particular significance for species which spawn on multiple occasions during the spawning season.

Cayre and Laloe (1986) suggest that the gonad index of female fish should be related to a quantitative histological or cytological factor in order to reflect the stage of sexual maturity. These authors state that the diameter of the most advanced mode of oocytes is the quantitative cytological factor most frequently used for female fish (a view shared by West, 1990). From their investigation of skipjack tuna, *Katsuwonus pelamis*, these authors conclude that, for a given maturity stage, the gonad index showed such variation that it did not permit a clear distinction of maturity stages and was not regarded as a reliable measure of maturity stage for in this species. These authors propose the concept of a critical value of gonad index (critical G.I.) after which there is no relationship between gonad index and oocyte diameter (d_{95} = lower size limit of the diameter of the most advanced 5% of oocytes). Having reached this critical G.I. (= 35 for this species) spawning is regarded as being imminent. These authors assert that the critical G.I. value enables distinction to be made between

individuals which are ready for reproduction, from those resting or beginning maturation, or those in post-spawning stages of reproduction.

The data presented in Figure 4.21 for *Latris lineata* shows a similar situation to that presented by Cayre and Laloe (1986) for *Katsuwonus pelamis*. In these data for *Latris lineata* oocyte diameter was determined from histological samples, as described in section 4.2.2. It is not possible to deduce a precise critical G.I. for *Latris lineata*, as more data must be collected to fill in the interval during which mean diameter of the most advanced oocytes increases from about 300 μm to 550 μm. This value is however suggested to be approximately 25, as all but one fish (G.I.= 18.26) with a G.I. above this value can be regarded as having been in a spawning condition, as determined by histology. Histological studies show that this sole exception was a spent fish retaining vitellogenic oocytes in an early state of resorption. This indicates that the gonad index of spent females may fall below the critical G.I. value suggested.

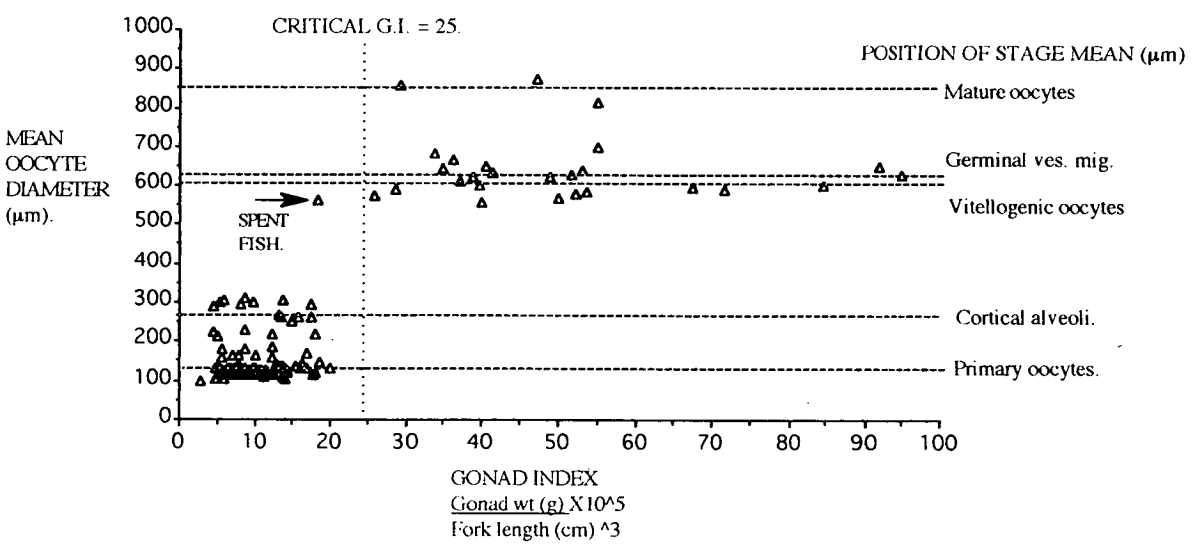


Figure 4.21 The relationship between the gonad index and the mean diameter of the 10 largest oocytes of *Latris lineata*, as determined by a histological study.

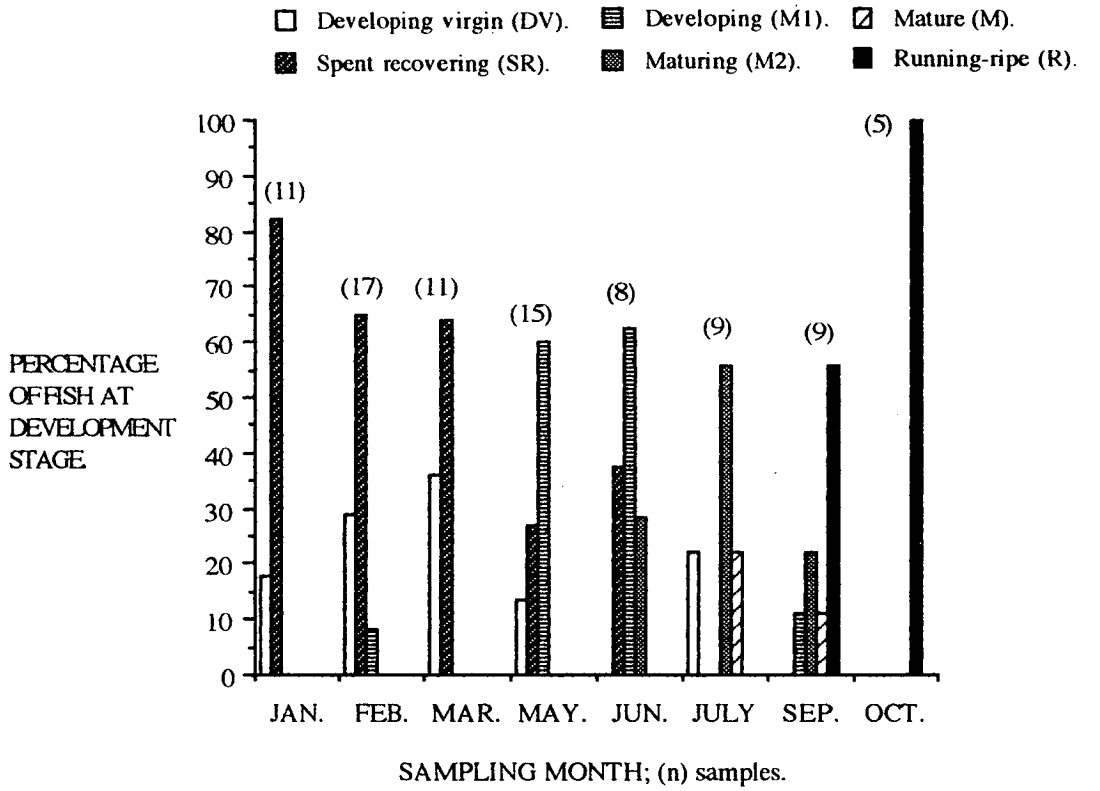
Although these data are incomplete the obvious separation apparent in Figure 4.21 supports the cautious use of a critical G.I. > 25 to distinguish female *Latris lineata* in an advanced stage of maturation, while there is a far from conclusive indication that spawning fish with G.I.< 25 may represent those in a spent condition.

A preliminary interpretation of the annual pattern of gonad development in male and female *Latris lineata* was conducted (Figures 4.22a and 4.22b) by arranging the maximum stage of gonad development data in chronological order (Appendix 13), based on sampling month. Samples sharing common months were combined and sampling year was ignored. Immature male fish (N=6) are excluded from the data (Figure 4.22a) as these fish could not having developed to a stage of maturation which would allow them to participate in spawning. No immature female fish were recorded in this study (section 4.4.2.1). As developing virgin females and spent recovering females are expected to undergo the same process of oocyte development, no distinction has been made between virgin and mature fish in the assessment of stages of gonad development presented in Figure 4.22b.

These data confirm that the spawning season of *Latris lineata* extends from September and the limited number of fish sampled on 4 October 1991 (n=10) can only suggest that spawning continues into this month. Male fish in particular (100%) were running-ripe in October, while females sampled during the spawning season (20 Sept.1990; 29 Sept. 1990; 4 Oct. 1991) showed evidence of having previously spawned, as post ovulatory follicles were identified in 22 (61.1%) of 36 samples examined.

Two way ANOVA comparing the presence of post-ovulatory follicles and stage of gonad development with both mean oocyte diameter and mean gonad index showed no significant (P> 0.05) interaction between the dependant variables (Appendices 14A, 14B and 14C). This result suggests that the presence of post-ovulatory follicles is not

a.



b.

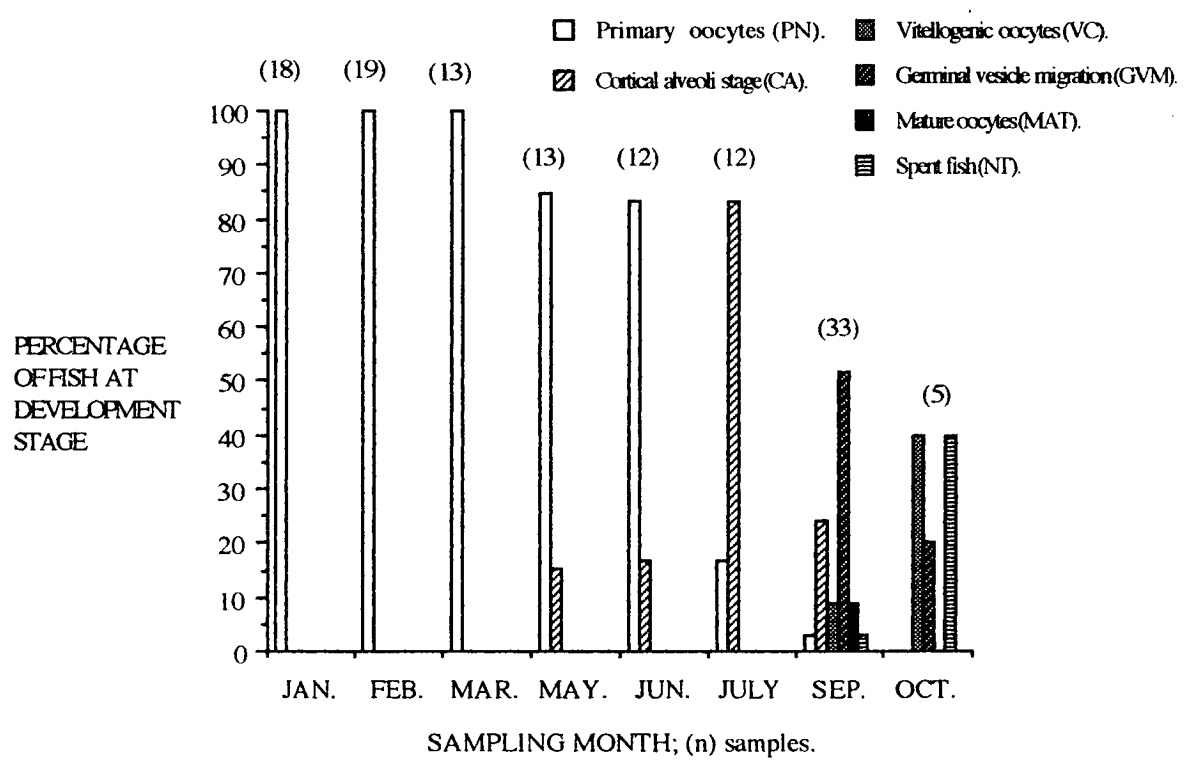


Figure 4.22 Annual pattern for stages of gonad development in *Latris lineata* as determined by histological analysis. (Data arranged in monthly chronological order, combining samples from common months and ignoring years.) **a.** Male fish (n=85). **b.** Female fish (n=125).

restricted to any stage of oocyte development (Figure 4.23) during the spawning season. This observation indicates that there is a replenishment of oocytes which occurs following the periodic ovulation and spawning of mature oocytes.

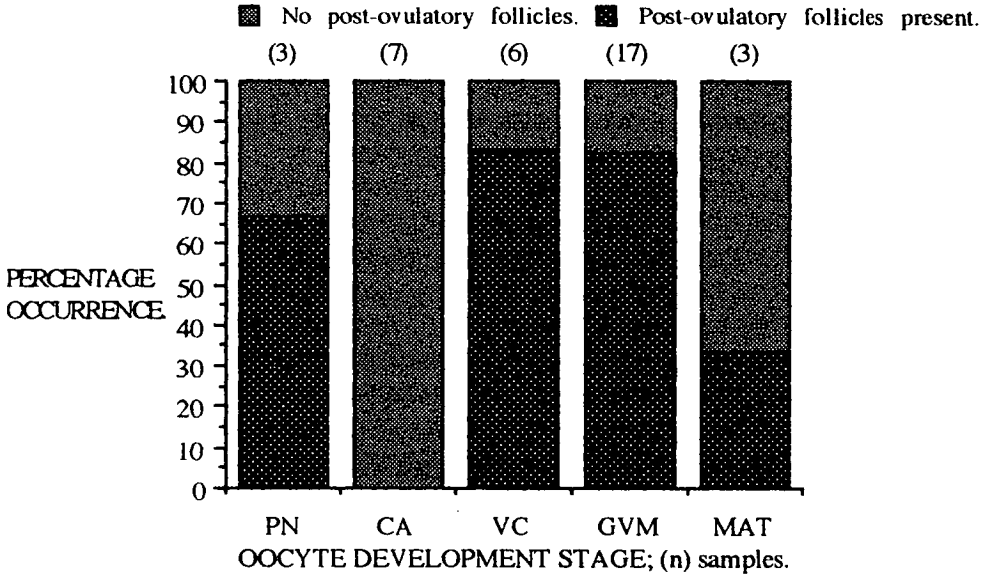


Figure 4.23 Percentage occurrence of post-ovulatory follicles for each oocyte development classification in ovaries of *Latris lineata* sampled during the spawning season.
(20 Sept. 1990; 29 Sept. 1990, 4 Oct. 1991; n=36).

The presence of post-ovulatory follicles among primary stage oocytes (Figure 4.23) indicates that these fish (N=2) were spent, while their presence together with oocytes at latter stages of development (VC, GVM, MAT) provides evidence that female *Latris lineata* spawn on more than one occasion. Munehara and Shimazaki (1989) use the presence of empty follicles (precursors to post-ovulatory follicles) to distinguish early spawning and maturing phases of reproduction identified for the multiple spawning masked greenling, *Hexagrammos octogrammus*. In both of these phases the most advanced oocytes are at the migratory nucleus stage, however empty follicles are only found in ovaries at the early spawning phase of reproduction. These authors suggest that multiple spawning in this species involves the repeated, intermittent maturing of clutches of oocytes each of which are subsequently spawned leaving empty follicles.

The pattern of oocyte development in teleosts (section 3.9) can be classified into the three categories based on their oocyte size distribution (Wallace and Selman, 1981; De Vlaming, 1983). As described in section 4.2.2, the diameters of 50 oocytes were measured from histological samples prepared from ovaries of *Latris lineata*. Large oocytes were targeted but all developing oocytes within the frame aquired for image analysis were measured. This selection criterion ensured that the range of developing oocytes found in each sample was represented. The distribution of all oocyte diameters measured from spawning female *Latris lineata* (largest oocytes at the CA stage or more developed) sampled during September 1990 is presented in Figures 4.24a - 4.24e. Figure 4.24a (all oocytes measured) shows that the distribution for oocyte diameters is continuous during vitellogenesis and germinal vesicle migration classification stages but becomes discontinuous with the onset of final oocyte maturation. This observation indicates the operation of group synchronous oocyte development (Asano and Tanaka, 1989).

When samples from individual fish are grouped on the basis of the maximum stage of oocyte development present, the result reveals that for females at the cortical alveoli stage the frequency distribution for oocyte diameters is uni-modal (Figure 4.24b). In fish with vitellogenic stage ovaries three modes of developing oocytes can be discerned (Figure 4.24c). When the mean oocyte diameters are used as identified previously in this chapter, it can be deduced that the first of these three modes approximates cortical alveoli stage oocytes, the second mode represents oocytes which have commenced vitellogenesis, while the third mode represents oocytes that have completed vitellogenesis.

Fish in which the maximum stage of oocyte development is identified as the germinal vesicle migration stage show a frequency distribution for oocyte diameters with less distinct modes (Figure 4.24d). As these data represent 17 fish it is asserted that although the most advanced mode (common to all fish) remains defined, the preceding

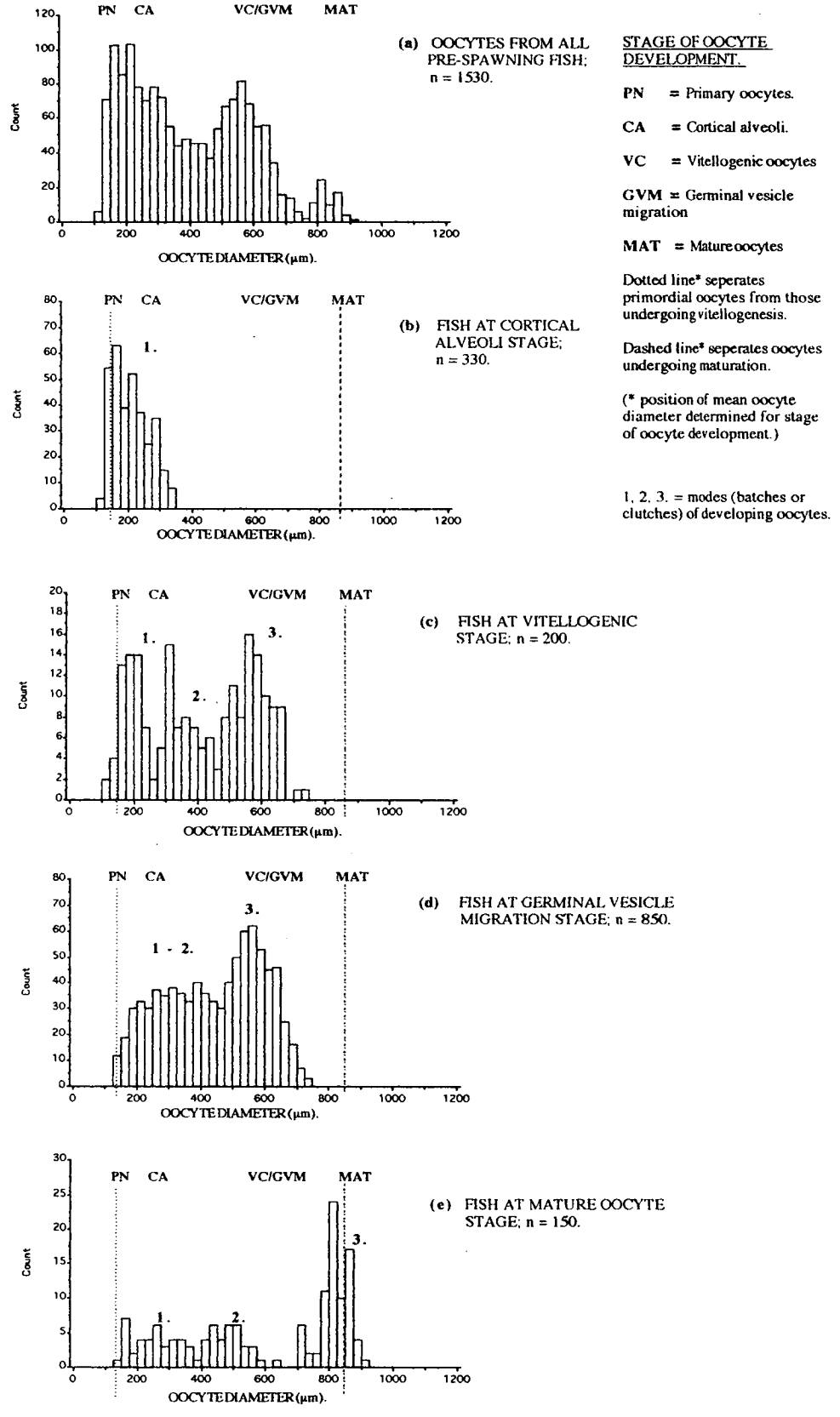


Figure 4.24a Frequency distribution of oocyte diameters from all mature female *Latris lineata* sampled in the 1990 spawning season (20 and 29 September).

Figures 4.24 b - 4.24e Frequency distribution of oocyte diameters from the same mature female *Latris lineata*, separated on the basis of maximum stage of oocyte development.

modes (discernable in samples from individual fish) are disguised by this composite data because the progress of these modes, through the continuum of vitellogenesis, is not synchronised between individuals. De Vlaming (1983) supports this view by acknowledging that pooling of data from several ovaries may obscure the actual pattern of development if there is not total synchrony of females within the sampled population.

Females identified as having mature stage oocytes also display a frequency distribution for oocyte diameters which is tri-modal (Figure 4.24e). Following the mature mode of oocytes is a mode in mid-late vitellogenesis together with another mode leaving the cortical alveolus stage and entering vitellogenesis.

Young *et al.* (1987) use the existance of a bi-modal distribution of oocytes from mature female pennant lightfish, *Maurolicus muelleri*, as evidence for multiple spawning in this species. Alternatively Gunn *et al.* (1989) use the development of a single batch of oocytes each season as a evidence for total spawning in blue grenadier, *Macruronus novaezelandiae*. Wallace and Selman (1981) report that three clutches of developing oocytes are discernable every evening in the medaka, *Oryzias latipes* and state that multiple clutches (more than two) have been noted in the ovaries of several other multiple spawning teleosts. Cayre and Farrugio (1983) report that four modes of oocytes can be identified in the maturing ovaries of skipjack tuna, *Katsuwonus pelamis*. Mayer *et al.* (1990) maintain that the pattern of oocyte development in sea bass, *Dicentrarchus labrax*, justifies the classification of this species as being group-synchronous based on oocyte size frequency diagrams in which at least two clutches can be distinguished at one time. It is proposed that during spawning of this species several discernable clutches are recruited from a large heterogeneous population of secondary oocytes.These authors conclude that this species is a 'fractional spawner' spawning a number of discrete clutches in quick succession with each successive clutch containing fewer oocytes.

Asano and Tanaka (1989) contend that the coexistence of distinct modes of oocytes at different stages of development provides proof of multiple spawning and that the overlapping of the gonad index of ovaries during the vitellogenic and mature stages of oocyte development provides further proof of multiple spawning. If the views of these authors is accepted, it can be concluded that the presence of three modes in the frequency distribution of oocyte diameters for *Latris lineata* during the spawning season and the overlapping of the gonad index values recorded at this time, provides evidence for multiple spawning in this species.

Wallace and Selman (1981) contend that in the daily spawning medaka, *Oryzias latipes*, there is a corresponding daily recruitment of a new clutch of oocytes into vitellogenesis. The existance of the three clutches of oocytes discernable in the ovaries of this species is explained by the two-day period required to complete the process of vitellogenesis. It is proposed that a similar situation may explain the three modes observed in the frequency distribution of oocyte diameters for *Latris lineata*, during the spawning season. Asano and Tanaka (1989) and Munehara and Shimazaki (1989) present diagrammatic explanations for the dynamics of the oocyte development process occurring in Japanese common mackeral, *Scomber japonicus* and masked greenling, *Hexagrammos octogrammus*, respectively. Both these species are described as multiple spawning fish. They display group synchronous oocyte development which allows them to serve as models from which the diagrammatic representation of the pattern of oocyte development in *Latris lineata* presented in Figure 4.25 has been developed.

The dynamics of the oocyte development model for *Latris lineata*, presented in Figure 4.25 are based on observations of the frequency distribution of oocyte diameters for individual fish together with the compiled distributions presented in Figures 4.24a-4.24e. This model suggests that discrete clutches of oocytes are periodically recruited into vitellogenesis. It is uncertain whether oocytes are recruited from a reserve

population at the primary or cortical alveoli stages of oocyte development. To ascertain conclusively the answer to this question all oocytes in each section or a sub-sample of ovarian tissue, would have to be measured. It is proposed that the presence of discernable modes of oocytes at, or about the cortical alveoli stage of development in females with vitellogenic (Figure 4.24c) and mature stage oocytes (Figure 4.24e) indicates that clutches of oocytes are being recruited from a reserve population of primary stage oocytes within the ovaries of *Latris lineata*.

A number of different recruitment tactics have been adopted by teleosts. In European sea bass, *Dicentrarchus labrax*, a population of primary oocytes is recruited into vitellogenesis during the period prior to the spawning season. During the spawning season successive clutches are recruited from this heterogeneous population of vitellogenic oocytes, however more than 90% of oocytes in the ovaries remain at the primary stages of development and do not appear to enter vitellogenesis in the current spawning season (Mayer *et al.*, 1990). Hay (1985) proposes that a single batch of oocytes is recruited into vitellogenesis from primary, "resting" oocytes during early stages of annual oocyte development in *Clupea harengus pallasi*. Wallace and Selman (1981) assert that in the Sticklebacks *Gasterosteus aculeatus* and *Apeltes quadracus*, injection of human chorionic gonadotrophin (HCG) causes recruitment of oocytes into vitellogenesis regardless of the stage of development of the leading clutch of oocytes. These authors contend that the same endocrine signal that elicits final oocyte maturation and ovulation is responsible for recruiting a new clutch of oocytes to replenish the ovary. It is apparent that teleosts displaying group-synchronous oocyte development show species specific strategies in which recruitment of replacement oocytes may be directly from oogonia, or from a population of oocytes 'arrested' at the end of the primary growth phase (De Vlaming, 1983).

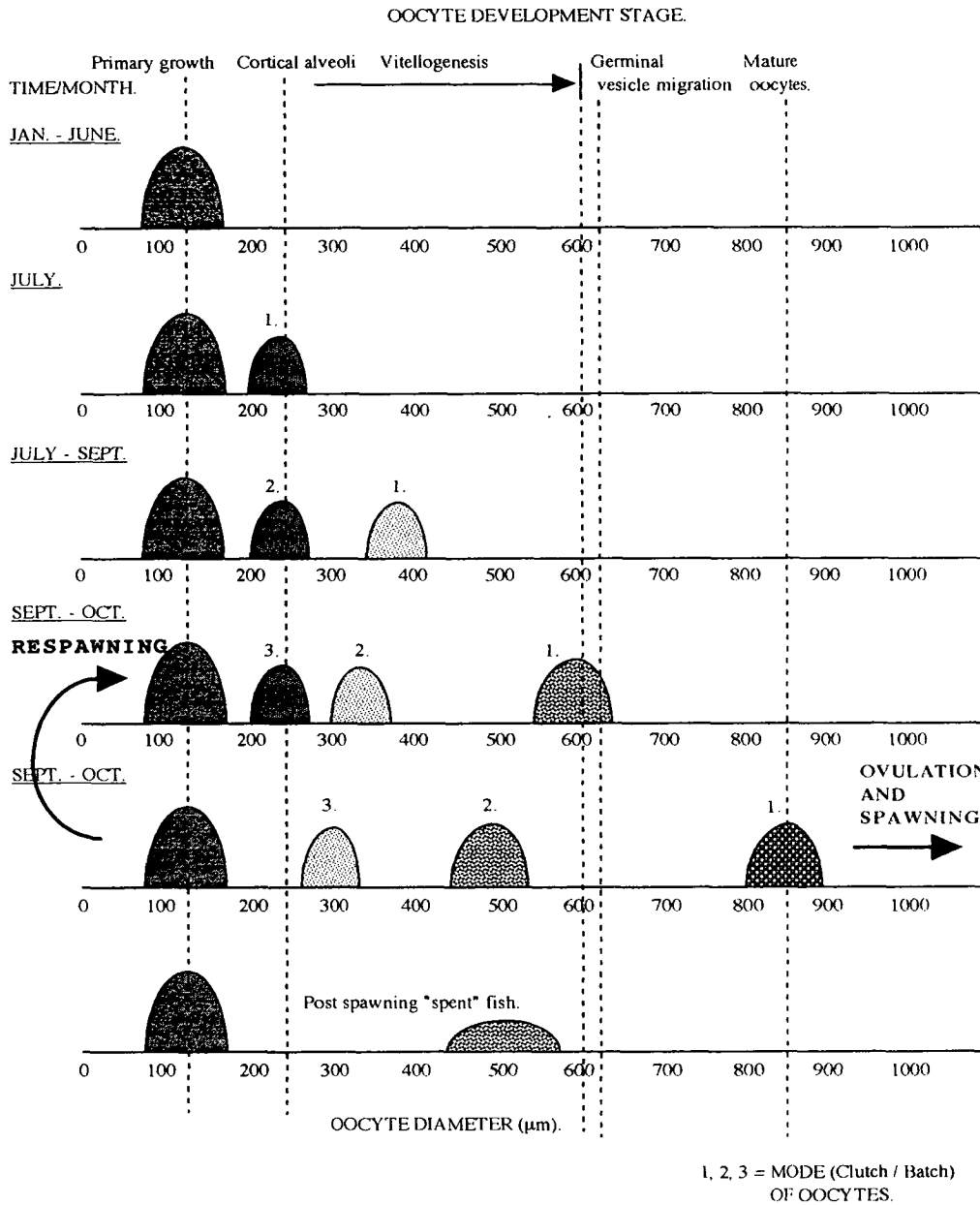


Figure 4.25 Diagrammatic representation of the pattern of oocyte development followed in *Latris lineata*.

It can be concluded from the findings presented in this section that *Latris lineata* is a gonochore which spawns on multiple occasions during the spawning season. The pattern of oocyte development presented for this species is best categorised as being group - synchronous with discrete batches of oocytes being spawned periodically during September and October each year. This conclusion is supported by the observations presented:

a The presence of post-ovulatory follicles together with vitellogenic and maturing oocytes;

b The frequency distribution for oocyte diameters becomes discontinuous with the onset of final oocyte maturation;

c The frequency distribution for oocyte diameters displaying three modes of oocytes involved in secondary development; and

d Overlapping of the gonad index during vitellogenic and mature stages of oocyte development.

The number of batches and frequency of spawning for each female can not be shown from this data.

4.6 Age and size at first maturation for *Latris lineata.*

The age and size of *Latris lineata*, at first maturation has not been reported. This information is of some importance to any effort directed at raising prospective broodstock. As shown in section 2.2, the fish sampled in this study had a restricted size range between 42.5 cm FL and 85.0 cm FL (Appendix 5). The samples studied excluded a considerable section of the life cycle of *Latris lineata*, during which early stages of gonad organisation may have occurred. Consequently immature *Latris lineata* are poorly represented in this study with only six immature males and no (zero) immature females identified.

Fish sampled in this study were captured at different months and in different years requiring the fork length and age at sampling to be extrapolated to a common reference date. This was done to allow comparisons to be made between all fish sampled and to present an age and fork length for each fish at this common time. From fork length measurements the age of all individual fish at the time of sampling was calculated using the relationships derived in section 2.3.

$$\text{Age (Years)} = 533.95 - 657.05 \times \text{Log}_{10}\,\text{F.L.} + 204.06\ \text{Log}_{10}\,\text{F.L.}^2$$

$$\text{Fork length (cm)} = 10.345 + 48.451 \times \text{Log}_{10}\,\text{Age.}$$

An adjustment equal to the fraction of a year remaining until the coming seasonal birth date (1 October) was then added to give all fish sampled a predicted age at a common time. The corresponding fork length of each fish at this common time was then calculated (Appendix 6). No data has been rounded up or down during manipulations. However, final results can be rounded to the common annual birthdate on 1 October.

4.6.1 Identification of *Latris lineata* approaching first maturation.

Of particular importance with respect to the description of first maturation is the identification of developing virgins of both sexes. Morrison (1990) uses the presence of spermatocytes to differentiate mature from immature male *Gadus morhua* . Davis (1982) relies on differences in the external appearance (degree of furrowing, size, translucence, opacity) to discriminate immature, developing and recovering spent male barramundi, *Lates calcarifer*. This author used the percentage of males at different maturity stages for each 50mm length class during months of highest gonadal activity, to identify the length at first maturity in this species. Asano and Tanaka (1989) also used length classes but employed monthly gonad index values to determine the size at maturity for *Scomber japonicus*.

A number of ovarian features are used to identify females that have previously spawned. The extent of vascularization and the presence of considerable connective

tissue in ovarian sections is used by Kjesbu (1987) to indicate that female blue whiting, *Micromesistius poutassou*, has previously spawned. The same author (1991) confesses that subjective, macroscopic features such as degree of vascularization, ovarian wall thickness, ovarian colour and the presence of unshed eggs, may result in incorrect distinction between immature and spent recovering female *Gadus morhua* which are greater than 40cm total length (<40 cm fish are immature). The ovarian wall of immature *Gadus morhua* is thin, giving the ovary a translucent appearance while the ovaries of mature females are more opaque due to the wall being thicker (Morrison,1990).

As the spawning season approaches, the onset of maturation in females can be identified by the occurrence of vitellogenic oocytes. Hay (1985) identifies two groups of oocytes in the ovaries of *Clupea harengus pallasi* . Mature females have a group of large maturing oocytes (>150μm) and a group of smaller resting oocytes (<150μm) while immature females have only resting oocytes. Kjesbu and Kryvi (1989) conclude that the presence of the circum-nuclear ring (suggested to be homologous to Balbiani bodies) seen in oocytes of *Gadus morhua*, indicates that the specimen will spawn in the coming season. This feature precedes the formation of cortical alveoli, the appearance of which marks the maturing of the gonad (Kjesbu, 1987). The presence of cortical alveoli can thus be used to differentiate mature and spent recovering females from immature fish, however their use is limited to the period following their occurrence.

The low representation of immature *Latris lineata* during the spawning season suggests that all but the six immature male fish sampled reach maturity within the size range represented in this study. All immature, developing virgin and recovering spent male *Latris lineata* were used to identify size and age at first maturity. The microscopic and macroscopic descriptions presented in sections 4.3 and 4.4

respectively, allow for distinctions to be made between these three classifications of maturity.

Analysis of size and age at first maturity for female fish was restricted to samples collected between the months of January and June (inclusive) each year. This interval encompasses the period in which developing virgin females could be identified (prior to the onset of vitellogenesis) and to allow analysis of differences in gonad index of classifications which is not significantly different ($P > 0.05$) between these months, when maturity stage is not considered (Table 4.1). Distinction between these developing virgins and spent recovering females based purely on stage of oocyte development can not be made during this interval, as a both categories follow a common sequence. For this reason the presence of atretic oocytes observed in ovarian sections throughout these months, was used as the definitive feature to identify fish which had spawned previously.

Atretic oocytes are commonly seen visually as brown specks in the ovarian tissue of spent recovering ovaries in *Latris lineata* (Figure 4.27a) throughout the pre-spawning, recovery period. Atresia most commonly occurs during vitellogenesis and post-ovulatory phases of maturation (Bromage and Cumaranatunga, 1988). Saidapur (1978) refers to these structures as atretic follicles or "corpora atretica" and suggests that they arise from pre-ovulatory follicles, in which the phagocytic granulosa cells subsequently digest and dispose of yolk and finally degenerate themselves. The persistence of atretic oocytes during non-vitellogenic stages of oocyte development in *Latris lineata* is indicative of females which have previously spawned, with these structures representing remnants of vitellogenic oocytes which did not mature. Microscopically atretic oocytes in *Latris lineata* appear as brown pigmented bodies (Figure 4.27b). These definitive structures were used to classify all histological samples as being from recovering spent (atretic oocytes present) of developing virgin (no atretic oocytes present) females.

4.6.2 Age and fork length of *Latris lineata* at first maturity.

The fork length and age (adjusted to 1 October) of individual male and female *Latris lineata* was used, together with the classification criteria described, to prepare the data (Appendices 15A and 15B) presented in Table 4.4. From these data it can be seen is that the minimum age at which male *Latris lineata* first mature is 8 years (7.8 years rounded to nearest year; 53.4 cm FL), while female *Latris lineata* can commence first maturation when they reach 5 years of age (43.8 cm FL). This age and size at first maturity is comparable with that of the only other description of first maturation for a species of the Latridae family. Francis (1988) reports that in New Zealand Blue moki, *Latridopsis ciliaris*, reach first maturity at about 40 cm and 5-6 years of age.

Table 4.4 Age (years) and fork length (cm) of male and female *Latris lineata* for maturity classifications used to determine first maturation for both sexes.

MATURITY CLASSIFICATION	SEX	FEATURE	Mean	+/- S.D	Min.	Max.	Range	N
IMMATURE	MALE	AGE (years)	7.3	1.7	5.7	10.1	4.4	
		FORK LENGTH (cm).	51.6	4.7	46.7	59.0	12.0	**6**
DEVELOPING VIRGIN	MALE	AGE (years)	9.2	1.1	7.8	11.8	4.0	
		FORK LENGTH (cm).	56.9	2.5	53.4	62.3	8.8	**15**
	FEMALE	AGE (years)	7.0	1.2	4.9	9.3	4.4	
		FORK LENGTH (cm).	50.8	3.7	43.8	57.3	13.5	**28**
SPENT RECOVERING	MALE	AGE (years)	10.9	2.2	6.8	16.1	9.3	
		FORK LENGTH (cm).	60.1	4.5	50.7	68.8	18.1	**34**
	FEMALE	AGE (years)	11.7	4.5	6.5	25.5	19.0	
		FORK LENGTH (cm).	60.8	7.4	49.8	78.5	28.7	**46**
						Total N =		**129**

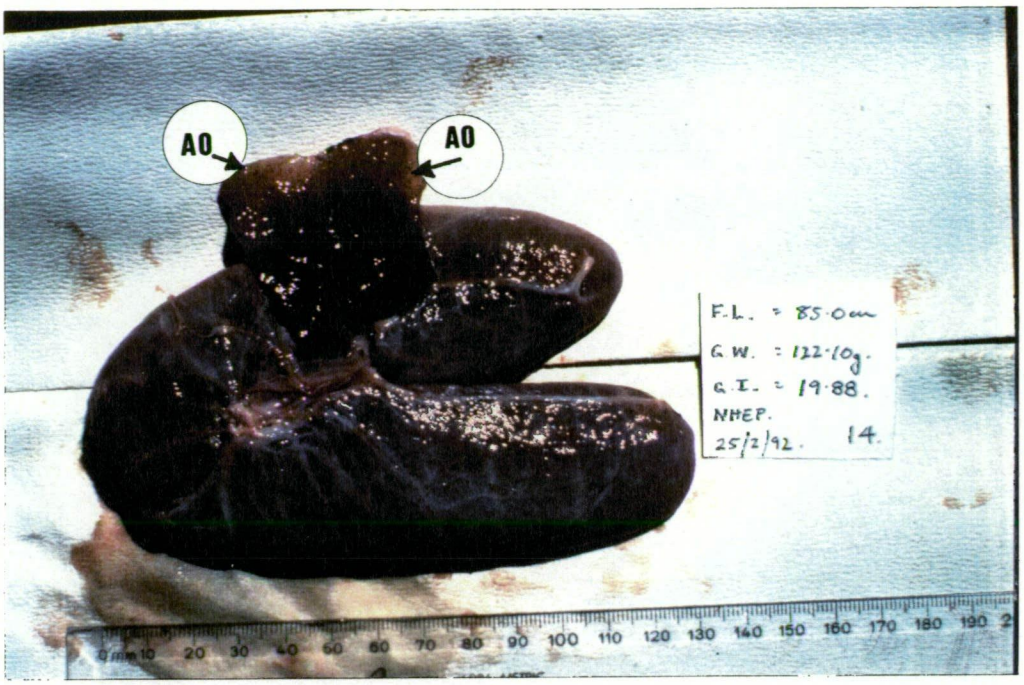


Figure 4.27a Recovering spent ovary of *Latris lineata* showing atretic oocytes (AO) visible as brown specks within the ovarian tissue.

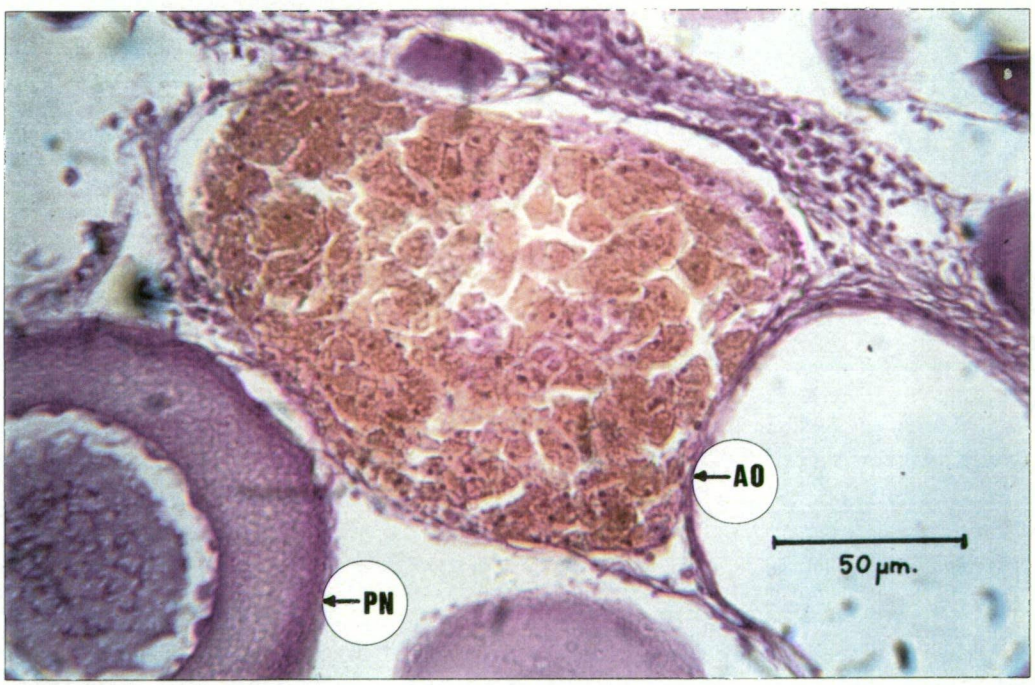


Figure 4.27b Cross section (X 800) of atretic oocyte in ovarian sample from *Latris lineata* (71.0 cm F.L; G.I. =12.96; Gonad weight = 46.4g) sampled 25 May, 1990 showing characteristic orange-brown pigmentation.

One way ANOVA (Appendices 15C and 15D) for developing virgin male and female *Latris lineata* shows that there is a significant difference ($P< 0.05$) in both the mean age and mean fork length at which the two sexes commence maturation. Male *Latris lineata* first mature on average 2.2 years (mean age at first maturity = 9.2 +/- 1.1 years) later than females (mean age at first maturity = 7.0 +/- 1.2 years). This conclusion is supported by the absence of immature females from these samples while six immature males were sampled within the same population of *Latris lineata*.

In both sexes there is a transitional interval of growth (age and fork length) in which both developing virgin and mature may fish occur. When the age extremities of this period are adjusted to whole years it can be seen that in both sexes this period extends for approximately four years after which time only mature male and female fish are found. All male fish larger than approximately 62 cm (12 years of age) are mature, while all females larger than approximately 57 cm (9 years of age) are mature. In females the range of sizes represented by this transitional interval is larger (13.5 cm compared to 8.8 cm for males) which may be explained by the faster relative growth rate of females which are younger (smaller) during this period.

Comparison between females with atretic oocytes (spent recovering, mature fish) and those females without atretic oocytes (developing virgins approaching first maturation) (Appendix 16A) using one way ANOVA (Appendix 16B) reveals a significant difference ($P<0.05$) for gonad index values. The mean gonad index of developing virgins is 7.02 +/- 1.78 while that of mature fish is 11.87 +/- 3.59, which reflects the larger proportional size of ovaries from mature fish. This observation is evident in Figure 4.28 which shows the overlap between the size (fork length) of developing virgin and mature fish.

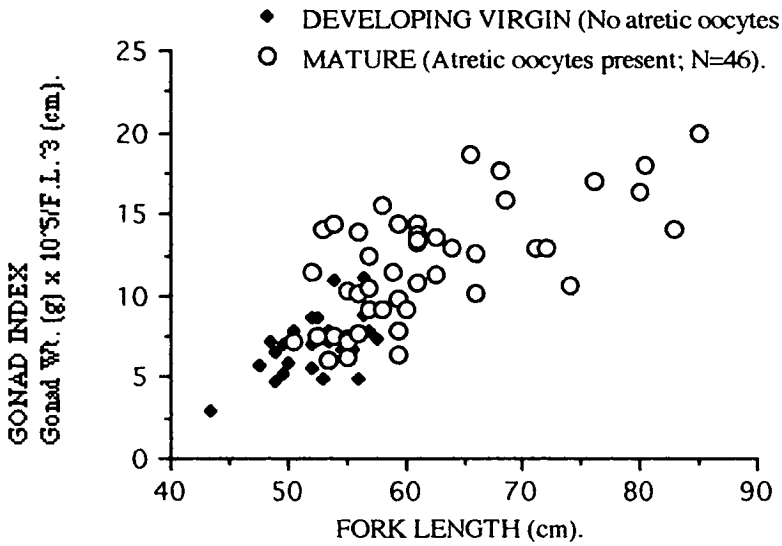


Figure 4.28 Relationship between fork length and gonad index of mature and developing virgin female *Latris lineata*.

4.7 Sex ratio for *Latris lineata*.

The ratio of females to males in *Latris lineata* for all sampling occasions combined are presented in Table 4.5. These ratios expressed as percentages for each 5cm size category are shown graphically in Figure 4.29. Comparison of sex ratios using chi-square (Table 4.5) shows that for young and first maturing *Latris lineata* the ratio of females to males is not significantly different . Only 4 (20%) of 20 fish over 70cm F.L. sampled were males, with no (zero) male *Latris lineata* larger than 80cm sampled. Although larger fish are predominantly females, the interval between 80.0cm and 84.5cm is the only interval in which this difference is significant ($P< 0.05$).

This observation suggests that as well as reaching first maturity at a older age, male *Latris lineata* do not reach the size achieved by their female counterparts. The implications of this situation with respect to the reproductive performance of the *Latris lineata* population sampled can only be speculated upon given the limited number of

fish sampled in this study. Berglund *et al.* (1989; unsighted in Trippel and Harvey, 1990) contend that females should predominate at low population densities at the risk of being in a spawning environment with insufficient males to fertilize their eggs. There is no significant ($P > 0.05$) imbalance in the overall sex ratio for *Latris lineata* however the imbalance between females and males in higher size categories suggests that there may be some selection operating which favours females and thus egg production, as size increases.

Table 4.5 Frequency counts, sex ratio and percentage of female to male *Latris lineata*, in relation to size (fork length).
*= significant $X^2_{0.05\,[1]} > 3.841$

INTERVAL (cm). FROM	TO	COUNT FEMALES	MALES	N	RATIO F : M	X	% FEMALE	% MALES
40	44.5	1	1	2	1 : 1.00	0.000	50	50
45	49.5	8	4	12	1 : 0.50	1.333	67	33
50	54.5	50	49	99	1 : 0.98	0.010	51	49
55	59.5	51	56	107	1 : 1.10	0.234	48	52
60	64.5	26	29	55	1 : 1.12	0.164	47	53
65	69.5	19	14	33	1 : 0.74	0.758	58	42
70	74.5	8	3	11	1 : 0.38	2.272	73	27
75	79.5	4	1	5	1 : 0.25	1.800	80	20
80	84.5	7	0	7	1 : 0.00	7.000*	100	0
85	89.5	1	0	1	1 : 0.00	1.000	100	0
	TOTAL	**175**	**157**	**332**	**1: 0.90**	**0.976**		

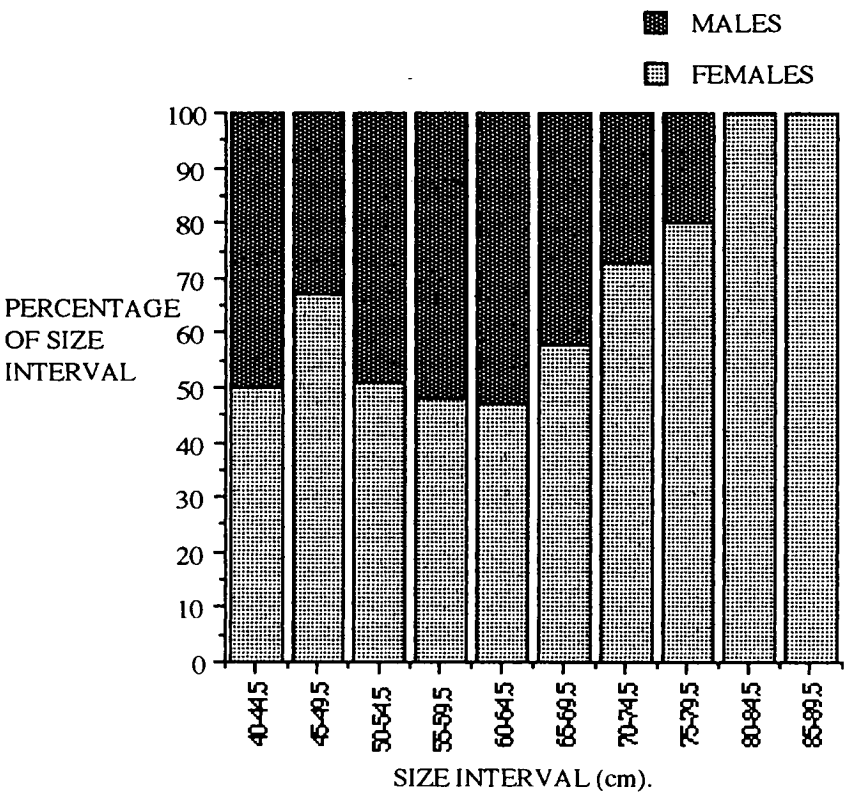


Figure 4.29 Percentage of female and male *Latris lineata* in relation to size (fork length) interval.

5 ENDOCRINE CONTROL OF REPRODUCTION AND HORMONE INDUCED SPAWNING OF TELEOSTS.

The regulation of the seasonal breeding cycles of temperate and cold water teleosts is important in optimizing the survival of the young-of-the-year (Scott, 1979; Crim, 1982; Hanyu and Razani, 1985) by synchronizing hatching with the availability of food (Billard *et al.*, 1981). Temperature, changes in temperature and photoperiod are the major physical environmental factors controlling the reproduction of temperate fish (Billard *et al.*, 1981, Lam, 1983). The spawning season, which represents the culmination of gametogenic events, is generally preceded by a far longer period of preparatory gonadal recrudescence (Scott, 1979, Crim, 1982). Environmental changes governing this gonadal recrudescence must be perceived, interpreted and transformed into functional signals in order to illicit a reproductive response. The responsibility for achieving this outcome in teleosts has been designated to a set of organs comprehensively described by Scott (1979) as the pineal-hypothalamus-pituitary complex. The participation of the gonad should not however be ignored, as suggested by the description of Crim (1982) who contends that the the timing of reproductive development of teleosts, in response to environmental conditions operates via the hypothalamo-pituitary-gonad axis.

5.1 Pineal involvement.

In teleosts the main pineal sac is housed in a recess within a partially depigmented, or translucent section of the cranium so that it is receptive to light from above (De Vlaming and Vodicnik, 1977; Scott, 1979). The pineal organ is recognized as a photo-receptor which receives information about the external photic environment from neurosensory cells of the pineal epithelium that are similar to the photosensory cells of the eyes (Fenwick, 1970; De Vlaming and Vodicnik, 1977; Hontela and Peter, 1980). The pineal organ in teleosts is also an endocrine gland with its secretory activity

influenced by light (De Vlaming and Vodicnik, 1977). Luteinizing hormone (LH) secretion in mammals is inhibited by the indolamine, melatonin (N-acetyl-5-methoxytryptamine) (Kezuka *et al* ., 1988) produced by the pineal organ (Fenwick, 1970). Histochemical and biochemical data show active indolamine metabolism in the pineal of teleosts (De Vlaming and Vodicnik, 1977). It is suggested that melatonin is a "time-keeping" hormone (Kezuka *et al.*, 1988) indirectly preventing ovarian and testis growth in fish by acting on certain hypothalamic centres of the brain which regulate gonadotropin secretion (Fenwick, 1970; De Vlaming and Vodicnik, 1977; Peter, 1981). Recent evidence has indicated an interaction between light and temperature in the control of pineal melatonin production in the white sucker, *Catostomus commersoni* and pike, *Esox lucius* (Bolliet *et al.*, 1993)

Production of melatonin by the teleost pineal increases as light intensity decreases. The resulting situation is that of pineal stimulation of gonadal activity during long photoperiods (reduced melatonin production) and suppression of gonad activity during short photoperiods (increased melatonin production).The effect of these variations in melatonin production is observed directly as changes in gonad recrudescence. In fish that spawn in spring the pineal organ stimulates gonadal recrudescence in the preceding months during which day lengths (photoperiods) increase (Vodicnik *et al.*, 1978; Hontela and Peter, 1980).

The inverse relationship between light intensity and the synthesis of melatonin by the pineal organ also causes daily (diurnal) fluctuations in plasma melatonin levels in common carp, *Cyprinus carpio.* These fluctuations vary in synchrony with the daily photoperiod (Kezuka *et al.*, 1988). This observation suggests an explanation for the diurnal fluctuations in the levels of pituitary and circulating gonadotropin shown in *Notemigonus crysoleucas* (De Vlaming and Vodicnik, 1977) and *Carassius auratus* (Vodicnik *et al.*, 1978; Hontela and Peter, 1980). Hontela and Peter (1978) and Peter (1982) contend that a significant daily cycle of serum gonadotropin is important in

stimulating gonadal activity and implicates the pineal organ as having an influence on daily cycles of gonadotropin secretion. Matsuyama *et al.* (1988) have shown that during the spawning season female red sea bream, *Pagrus major* exhibit diurnal fluctuation in plasma steroids 17β-estradiol and testosterone which accompany a diurnal ovarian rhythm of oocyte maturation. A similar diurnal fluctuation in the level of the maturation mediating steroid 17α,20β-dihydroxy-4-pregnen-3-one has been shown for the same species (Kagawa *et al.*, 1991).

5.2 Invovement of the hypothalamus-pituitary complex.

It is generally accepted that in teleosts the pituitary (hypophysis) is regulated by the the hypothalamus (Barnett *et al.*, 1982) and that the pituitary gland controls the activities of the thyroid and inter-renal glands, gonadal function and growth (Peter, 1970). The pituitary consists of a neurohypophysis and an adenohypophysis which can be divided into three parts, the rostral pars distalis, proximal pars distalis and the neurointermediate lobe. Gonadotropic (GtH) cells containing a heterogeneous population of secretory granules and globules, are usually found in the proximal pars distalis of the African catfish, *Clarias gariepinus* (DeLeeuw, 1985; Peute *et al.*, 1987). and along the periphery of pars distalis in winter flounder, *Pseudopleuronectes americanus* (Weil *et al.*, 1992). Gonadotrophs are scattered in the central and ventral parts of the posterior pars distalis in three sciaenid species, namely; Atlantic croaker, *Micropogonias undulatus*, spotted sea trout, *Cyanoscion nebulosus* and red drum, *Sciaenops ocellatus* (Yan and Thomas, 1991).

In higher vertebrates the hypothalamus regulates the anterior pituitary gland via hypothalmic releasing hormones secreted into pituitary portal vessels for transport to the adenohypophysis. There is interdigitation of the hypothalamus and the pituitary (pituitary stalk) in teleosts which allows this regulation to be achieved through direct innervation or paracrine (cell to cell) effects (Barnett *et al.*, 1982). Neurohormones,

such as gonadotropin-releasing hormone (GnRH), are important in regulating the reproductive system (Sherwood *et al.*, 1989).

Immunocytological techniques have been employed to investigate the distribution of GnRH in the brain and pituitary of teleosts. There is a lack of agreement between studies. However, results suggest that the nucleus lateral tuberis, telencephalon and preoptic region, are centers for GnRH cells in the brain of teleosts (Peter, 1982). In the pituitary of the African catfish, *Clarias gariepinus*, GnRH is located in nerve cells of the nucleus preopticus of the brain, hypothalamic axons and secretory granules close to the gonadotropic cells of the proximal pars distalis (Goos *et al.*, 1985).

5.2.1 Neuropeptides.

In teleosts a GnRH similar to the mammalian neuropeptide, luteinizing hormone releasing hormone (LHRH) has been demonstrated (Peter, 1982; Chang and Peter, 1983) and the mammalian LHRH decapeptide is biologically active in teleosts (Barnett *et al.*, 1982). More than 2000 analogues of mammalian LHRH ($pGlu^1$, His^2-Trp^3-Ser^4-Tyr^5, Gly^6, Leu^7-Arg^8-Pro^9-Gly^{10}-NH_2) have been synthesized. Biochemical studies have shown that teleost GnRH differs from mammalian LHRH due to the amino acid sequence of the primary structure (Crim *et al.*, 1988). Salmonid GnRH differs from mammalian GnRH in two amino acids (Trp-Leu) at the seventh and eighth positions (Sherwood *et al.*, 1983). A salmon-like GnRH has been demonstrated in other teleosts including trout, milkfish, mullet, goldfish and herring (Sherwood *et al.*, 1989). Two distinct LHRH related peptides are reported in codfish, *Gadus morhua* (Barnett *et al.*, 1982) and catfish (Sherwood *et al.*,1989). In addition, GnRH receptors have been identified as a single class of high affinity binding sites in cultured gonadotrophic cells from pituitaries of *Clarias gariepinus* (DeLeeuw *et al.*, 1988; unsighted in Sherwood *et al.*, 1989) and *Psuedopleuronectes americanus* (Weil *et al.*, 1992).

5.2.2 Neuroamines and neuroendocrine control of gonadotropin secretion.

Brain lesioning experiments using *Carassius auratus* suggest the existance of a gonadotropin-release inhibiting factor (GRIF). These experiments indicate the probable origin of GRIF in the anterior preoptic region from which it is carried by pathways in the lateral preoptic and hypothalamic regions to the pituitary stalk (Peter, 1982). The amine neurotransmitter (neuroamine) dopamine has been shown to act directly on gonadotropic cells of the pituitary to inhibit GtH release and directly modulate or block the action of GnRH in goldfish and catfish. This suggests the possible function of dopamine as a neuroendocrine regulator of reproduction (Peter *et al.*, 1986; DeLeeuw *et al.*, 1987).

The direct regulatory effect of dopamine on GtH cells of the pituitary is supported by observations that the dopamine receptor antagonist pimozide potentiates the release of GtH induced by analogues of LHRH (LHRH-a). Ovulation was induced in *Carassius auratus* by an injection of pimozide into fish which had previously been implanted with a LHRH-a pellet (Sokolowska *et al.*, 1984). Similarly, injection of pimozide 3 hours prior to injection of LHRH-a significantly increased serum GtH levels of *Carassius auratus*, compared to fish injected with pimozide or LHRH-a alone (Sokolowska *et al.*, 1985). Pankhurst *et al.* (1986) report that injection of pimozide accelerates oocyte development in walleye, *Stizostedion vitreum*, however it did not potentiate the induction of ovulation with LHRH-a. Pimozide greatly potentiates the effects of LHRH-a in catfish as it does for common carp and eel (DeLeeuw, 1985). The injection of a dopamine receptor antagonist (domperidone or pimozide) in combination LHRH-a or the teleost GnRH analogue [D-Arg^6, Trp^7, Lue^8, Pro^{9}NEt]-LHRH (sGnRH-a), has proven successful in inducing ovulation in a number of species of freshwater fish in China (Common carp, *Cyprinus carpio*; silver carp, *Hypophthalmichthys molitrix*; mud carp, *Cirrhinus molitorella*; bream, *Parabramis pekinensis*; grass carp, *Ctenopharyngodon idellus*; big head carp, *Aristichthys nobilis*;

black carp, *Mylopharyngodon piceus* and Loach, *Paramisgurnus dabryanus*) (Peter *et al.*, 1988). The combination of pimozide with both LHRH-a and sGnRH-a was effective in inducing ovulation in goldfish while LHRH-a and sGnRH-a alone were ineffective, although significant increases in levels of serum GtH were recorded following administration of the GnRH analogues (Sokolowska *et al.*, 1984; Peter *et al.*, 1987).

It is accepted that in teleosts, neurosecretory axons communicate with the pars distalis of the pituitary and their ends make synaptoid contact with gonadotropic cells in some species. There is evidence of two types of neurosecretory fibers in contact with gonadotropic cells of the pituitary and ultrastructural observations suggest these fibers originate in the nucleus lateral tuberis and nucleus preopticus of the brain (Peter, 1982). Peute *et al.* (1987) describe these two types of hypothalmic neurosecretory neurons as peptidergic (GnRH) and dopaminergic. These authors contend that there is increasing support for a dual neurohormonal control of gonadotropin secretion in teleosts. These authors provide evidence for the existence of GnRH and dopamine immunoreactive nerve fibers in synapse-like contact with GtH cells in pituitary of *Clarias gariepinus*. It is suggested that this intracellular mechanism allows dopamine to act directly to inhibit GnRH stimulated GtH release.

Omeljanuik *et al.* (1987) and Peter *et al.*(1987) propose the concept that dopamine inhibition of GtH release is receptor mediated in *Carassius auratus*. The mammalian dopamine D_2-receptor antagonist domperidone was tested together with other dopamine receptor antagonists (pimozide, haloperidol and spiperone) to investigate pituitary function in *Carassius auratus*. Results of investigations show that in this species, the receptors mediating GRIF activity of dopamine have characteristics consistent with mammalian D_2-dopaminergic receptors.

5.2.3 Gonadotropins.

The mammalian pituitary gland secretes two gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Both these glycoproteins are composed of two chemically distinct, covalently bound sub-units. The α sub-unit has a species specific amino acid sequence common to both gonadotropins while the β sub-unit is hormone specific (Burzawa-Gerard, 1982; Suzuki *et al.*, 1988a). It was initially suspected that teleosts possessed a single GtH responsible for the gonadal actions homologous with those attributed to the LH/FSH glycoproteins of mammals (Peter *et al.*, 1986; Swanson *et al.*, 1991). It is now accepted that there are two gonadotropic hormones produced by the pituitary of teleosts although results of early research provided some conflicting results.

Using affinity chromatography on concanavalin-A (Con A) Sepharose Idler and Ng, see below, isolated two types of GtH, unabsorbed GtH I (Con A I, low carbohydrate content) and absorbed GtH II (Con A II, high carbohydrate content) from pituitaries of American plaice, *Hippoglossoides platessoides*, winter flounder (Ng and Idler, 1978a), chum salmon (Ng and Idler, 1978b), and common carp (Idler and Ng, 1979). Con A II stimulated gonadal vitellogenesis and displayed chemical homology to LH and FSH. However, although Con A I stimulates in vivo vitellogin uptake by ovarian follicles, it does not display chemical homology to tetrapod GTHs. Whereas Con A II consists of two disimilar subunits, as is the case with LH and FSH; the subunit nature of Con A I has not been demonstrated (Suzuki *et al.*, 1988a; Nozaki *et al.*, 1990; Swanson *et al.*, 1991)

More recently two distinct pituitary GtHs (GtH I, GtH II) have been isolated from chum salmon, *Oncorhynchus keta*, (Suzuki *et al.*, 1988a,b,c), coho salmon, *Oncorrhynchus kisutch*, (Swanson *et al.*, 1991) and common carp, *Cyprinus carpio* (Van Der Kraak *et al.*, 1992). In these species GtH I and GtH II consist of two

disimilar subunits, with a common α-subunit and differing β-subunits. Both these GtHs display structural homology to tetrapod FSH and LH and it appears that the maturational GtH (Con A II) previously isolated by Idler and coworkers corresponds to GtH II isolated from chum salmon. The absence of GtH I from previous studies is attributed to it not being identified. It is concluded that GtH I is a novel GtH molecule with distinctly different chemical characteristics than GtH II (Swanson *et al.*, 1991).

Previous descriptions (section 3.4) established that vitellogenesis (oocyte growth) and maturation are major phases of teleost oocyte development. Latter information will confirm the significant dependance of each of these events on the steroid hormones 17β-estradiol and 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-diOHprog), respectively, produced by the ovary under the influence of GtH. Young *et al.*, (1983a, 1983b) and Kanamori *et al.*, (1988a) contend that GtH stimulated production of steroid hormones by ovarian follicles shifts from secretion of predominantly 17β-estradiol during vitellogenesis, to the secretion of 17α,20β-diOHprog during maturation. The production of these steriods is thus used in bioassay procedures to verify the activity of purified GtHs, and to determine the involvement of GtH I and GtH II during the process of oocyte development.

Ng *et al.* (1982) contends that GtH I has only vitellogenic activity while GtH II is maturational and is also active in vitellogenesis. More recently Suzuki *et al.* (1988b) have demonstrated that GtH I and GtH II from chum salmon, *Oncorhynchus keta*, both stimulated dose related *in vitro* production of 17β-estradiol by intact, mid-vitellogenic ovarian follicles from amago salmon, *Oncorhynchus rhodurus*. In a study on post-vitellogenic follicles, GtH II appeared to be more effective than GtH I in stimulating the production of 17α,20β-diOHprog. In addition, GtH II was found to be more potent than GtH I in stimulating both the release of the precursor 17α-hydroxyprogesterone (17α-HProg) by ovarian thecal layers, and the production of 17α,20β-diOHprog by granulosa layers, in the presence of 17α-HProg. *In vitro*

bioassays established that the production of 17β-estradiol and total androgens by juvenile ovarian and testicular tissue is stimulated by coho salmon GtH I and GtH II in a similar, dose dependant manner (Swanson *et al.*, 1991).

Using rainbow trout for *in vivo* and *in vitro* studies, Tyler *et al.* (1991) demonstrated that GtH I stimulated the uptake of vitellogenin into mid-vitellogenic follicles in a dose- dependent manner, while vitellogenin uptake by GtH II treated follicles was not enhanced above untreated control follicles. In these experiments both GtH I and GtH II stimulated the secretion of 17β-estradiol by the follicles however *in vivo* studies showed that GtH I, but not GtH II, significantly elevated the plasma level of vitellogenin. Van Der Kraak *et al.* (1992) state that both GtH I and GtH II from pituitaries of *Cyprinus carpio*, stimulate ovarian and testicular steroidogenesis, enhance ovarian sequestration of vitellogenin and induce oocyte maturation. Further support for the concept of a duality of GtHs in teleosts is provided by the pattern of synthesis for these hormones during reproductive development and the localization of GtH I and GtH II in the pituitary. Although the steroidogenic activities of GtH I and GtH II are similar when tested *in vitro*, the levels of these GtHs in the pituitary and the circulatory system change significantly during reproductive development (Suzuki *et al.*, 1988a; Swanson *et al.*, 1991). The level of GtH I in the plasma of coho salmon, *Oncorhynchus kisutch* increases during gametogenesis while the level of GtH II is low or not detectable. During final maturation and release of gametes (ovulation and spermiation) the level of GtH I in the plasma declines while the level of GtH II increases markedly (Swanson, 1991). In rainbow trout, *Oncorhynchus mykiss*, GtH I levels in the pituitary and plasma are higher than levels of GtH II during prematurational stages of vitellogenesis and spermatogenesis. During final reproductive maturation (spermiation and ovulation) GtH II is the dominant GtH which suggests that the periods of synthesis and secretion of the two GtHs differ during the reproductive season (Suzuki *et al.*, 1988c). This situation is of functional significance as GtH II is more potent in stimulating release of the maturational steroid

17α,20β-diOHprog, while GtH I stimulates the uptake of vitellogenin into developing oocytes (Suzuki *et al.*, 1988c; Tyler *et al.*, 1991)

An immunocytological study by Nozaki *et al.* (1990a) revealed that GtH I and GtH II are produced in distinctly different cells of the pituitary in rainbow trout and Atlantic salmon, *Salmo salar*. Apparently active GtH I cells are found in the pituitary of rainbow trout before the onset of spermatogenesis and vitellogenesis, while GtH II cells only appear at the onset of these stages of reproductive development. In an accompanying study Nozaki *et al.* (1990b) found only GtH I cells in the pituitaries of immature rainbow trout. The number of GtH I cells significantly exceeds the number of GtH II cells present during early gametogenic stages. However, significantly more GtH II cells than GtH I cells are found in mature fish just before spawning. These changes in the number of GtH I and GtH II gonadotrophs correspond to the fluctuations in the pituitary content and circulating levels of these GtHs, as reported by Suzuki *et al.* (1988b,1988c) and Swanson *et al.* (1991) (Nozaki *et al.*, 1990b).

It can be concluded from the available information presented that the existance of at least two distinct GtHs have been demonstrated, each located in separated pituitary gonadotrophs. The level of GtH I and GtH II in the pituitary and serum varies during reproductive development. Although both GtHs are steroidogenic, GtH I is primarily responsible for stimulating vitellogenin uptake (vitellogenesis) while GtH II is more potent in the induction of the maturational steroid 17α,20β-diOHprog.

5.3 Response to gonadotropins.

Vitellogenesis and final oocyte maturation in female teleosts is largely dependant on the action of ovarian steroids (Sakai *et al.*, 1988). Accepting the duality of teleost GtHs secreted by the pituitary gland, it is apparent that both GtH I stimulation of vitellogenesis and GtH II stimulation of final oocyte maturation are achieved indirectly

through stimulation of follicular steroidogenesis.

5.3.1 Stimulation of vitellogenesis and spermatogenesis.

Crim *et al*. (1975) describe the general pattern of plasma GtH levels for males and females of the salmonid species sockeye salmon, *Oncorhynchus nerka*, brook trout, *Salveinus fontinalis*, and brown trout, *Salmo trutta*. In males of these species GtH remains low or undetectable during early phases of gonadal recrudescence and the start of spermatogenesis with peak levels recorded in fish at spermiation. Female fish have low titers of GtH during early stages of ovarian development including vitellogenesis, during which time the level of GtH does rise, however a dramatic increase in GtH precedes ovulation. In both sexes plasma GtH levels were low during the time of the maximum rate of gonad development. Stacey *et al.* (1979) demonstrated the existence of an ovulatory surge in levels of GtH in goldfish induced to ovulate after an increase in water temperature. Serum GtH levels increased following transfer of fish to an "ovulation aquarium". Maximum levels of serum GtH were reached during the period of ovulation, after which serum GtH levels fell rapidly to pre-induction levels. The surge in gonadotropin secretion which accompanies the completion of vitellogenesis is believed to induce final oocyte maturation by stimulating the secretion of maturation-inducing steroids (Nagahama, 1983). Sakai *et al.* (1987) support this view. They suggest that increased plasma levels of GtH before spawning may trigger the follicular synthesis of 17α,20β-diOHprog in medaka, *Oryzias latipes*.

A common pattern for circulating levels of 17β-estradiol in response to GtH has been established in many teleost fishes. Levels of 17β-estradiol start to increase during the late preparatory period preceding spawning when rapid vitellogenesis occurs. Peak levels are reached during the pre-spawning period. Levels then fall precipitously prior to final oocyte maturation during the spawning period. This pattern is observed in the striped mullet, *Mugil cephalis* (Dindo and MacGregor, 1981) blue fish, *Pomatomus*

saltator, king mackeral, *Scomberomorus cavalla* (MacGregor *et al.*, 1981) catfish, *Heteropneustes fossilis* (Sundararaj *et al.*, 1982), amago salmon, *Oncorhynchus rhodurus* (Kagawa *et al.*, 1983), white sucker, *Catostomus commersoni*, (Scott *et al.*, 1984), medaka, *Oryzias latipes* (Sakai *et al.*, 1988) and sweep, *Scorpis lineolatus* (Dedual and Pankhurst, 1992). Matsuyama *et al.*, (1988) relating the pattern steroid fluctuations to the stages of oocyte development in the daily spawning red sea bream, *Pagrus major*. In this species plasma 17β-estradiol levels reach a peak in fish with pre-mature stage oocytes and rapidly decline under ovulating conditions.

Pankhurst and Conroy (1988) reviewed the work of several researchers and concluded that in the majority of teleosts, androgen levels remain low during gonadal recrudescence. Peak androgen levels occur before final oocyte maturation in females, and at the end of spermatogenesis in males, then fall rapidly at the start of these maturational phases of reproductive development. Variations on this general pattern do however occur. Testosterone levels increase when vitellogenesis is almost completed and persist until the end of the maturational phase in the catfish, *Heteropneustes fossilis* (Lamba et al., 1983). In amago salmon plasma testosterone levels follow a similar pattern to that of 17β-estradiol. However peak levels are maintained during the period of final oocyte maturation and ovulation (Kagawa *et al.*, 1983). Pankhurst and Conroy (1988) report that androgen levels in male and female orange roughy, *Hoplostethus atlanticus*, declined with time rather than with the start of final gamete maturation. This suggests that there may be another steroid produced which perhaps functions as a pheromone to synchronize spawning and which is responsible for the androgen profile observed in this species.

5.3.2 Stimulation of final oocyte maturation and spermiation.

Following vitellogenesis developing oocytes must undergo final maturation and ovulation prior to spawning. The steroid 17α,20β-diOHprog has been found to be the

most potent maturation-inducing steroid in studies conducted on a number of species of teleosts (Goetz, 1983). 17α,20β-diOHprog has been shown to promote *in vitro* oocyte maturation in rainbow trout, northern pike and goldfish (Jalabert, 1976), yellow perch, *Perca flavescens* (Goetz and Theofan, 1979), amago salmon (Young *et al.*, 1983b), medaka, *Oryzias latipes* (Sakai *et al.*, 1988), tropical catfish, *Clarias macrocephalus* (Suzuki *et al.*, 1989) and tobinumeri-dragonet, *Repomucenus beniteguri* (Zhu *et al.*, 1989). In addition to the maturational role of 17α,20β-diOHprog in female teleosts it has also been demonstrated that this steroid is effective in inducing *in vivo* spermiation in amago salmon and goldfish, which involves testicular hydration and a concomitant thinning of the semen. It is proposed that 17α,20β-diOHprog plays an important role in mediating GtH induced spermiation in teleosts (Ueda *et al.*, 1985; Sakai *et al.*, 1989).

A wide range of biologically active steroid metabolites can be synthesized by ovarian tissue during final oocyte maturation and ovulation. Three metabolites of 17α,20β-diOHprog which are produced by intact follicles of *Fundulus heteroclitus* were found by Petrino *et al.* (1993) to be as effective as 17α,20β-diOHprog in inducing *in vitro* GVBD. However, 17α,20β-diOHprog was the fastest acting and most potent. It is suggested that although 17α,20β-diOHprog is not the only active steroid produced by maturing follicles, it may play the major role during oocyte final maturation (Petrino *et al.*, 1993). In an *in vitro* study of rainbow trout oocytes, Canario and Scott (1988) identified four steroids which were equipotent to the reference steroid 17α,20β-diOHprog in inducing maturation.

No measurable changes in plasma levels of 17α,20β-diOHprog were recorded during final gamete maturation in male or female orange roughy, *Hoplostethus atlanticus*, which suggests that this is not the maturation-inducing steroid for this species (Pankhurst and Conroy, 1988). *In vitro* studies on oocytes of Atlantic croaker, *Micropogonias undulatus* have identified 17α,20β,21-trihydroxy-4-pregnen-3-one

(20β-S) as the major maturation-inducing steroid. In this study over ten times more 20β-S was produced by intact oocytes than 17α,20β-diOHprog (Trant and Thomas, 1989). Unpublished results reported by Kagawa *et al.*, (1991), suggest that 20β-S is a potent inducer of *in vitro* oocyte maturation in red sea bream. However 17α,20β-diOHprog also plays a major role during this process, in this species. Dedual and Pankhurst (1992) measured an increase in plasma 17α,20β-diOHprog during vitellogenesis in the sweep, *Scorpis lineolatus*, however maximum levels were low and decreased prior to maturation. This suggests that this steroid may not be the maturation inducing steroid in *Scorpis lineolatus*. The steroids 3β,17α-20α-trihydroxy-5β-pregnane (3β,17,20α-P-5β) and 17α,20α-dihydroxy-4-pregnen-3-one (17α,20α-OHprog) were found in high concentrations in the plasma of mature female and male dab, *Limanda limanda*, which indicates that in this, and other species, 17α,20β-diOHprog is not involved in oocyte final maturation (Canario and Scott, 1989).

In view of such findings Petrino *et al.* (1993) advance the concept that it may be more appropriate to consider the final maturation of oocytes as being induced by an overall "endocrine milieu" rather than being controlled by a single maturation-inducing steroid. Canario and Scott (1989) contend that the adoption of other maturation-inducing steroids may be associated with their pattern of spawning as most of these alternative species are serial spawners. Petrino *et al.* (1993) propose that the possible collaboration of 17α,20β-diOHprog and its biologically active metabolites suggested for *Fundulus heteroclitus*, may apply generally to fractional or multiple spawners.

5.3.3 Production of steroids.

The production of 17β-estradiol, testosterone and maturational steroids has been attributed to follicle (theca and granulosa) and interstitial cells of female and male teleosts respectively (Nagahama *et al.*, 1982; Fostier *et al.*, 1983). The changes in

levels of plasma testosterone during vitellogenesis appears to be associated with the participation of this steroid as a precursor in the metabolic pathway for the synthesis of 17β-estradiol (Young *et al.*, 1983a). From the use of specific radioimmunoassays to determine the levels of 17β-estradiol and testosterone in the incubation medium of separated thecal and granulosa cell layer cultures, Kagawa *et al.* (1982) advanced the concept of a "two-cell-type" model for the production of 17β-estradiol in teleosts. Based on results obtained with amago salmon this model postulates that special cells of the thecal layer are the site of the numerous biosynthetic steps involved in the production of androgens which are aromatized through a few biosynthetic steps to 17β-estradiol within cells of the granulosa layer. This model is supported by results of investigations by Kagawa *et al.* (1982) and Kanamori *et al.* (1988a) in which isolated thecal layers from amago salmon follicles, incubated in the presence of GtH, produced large amounts of testosterone and very little 17β-estradiol. Cultures of isolated granulosa layer, in the presence of exogenous testosterone, produced 17β-estradiol independent of the addition of gonadotropin while thecal layers produced little 17β-estradiol. This suggests that all that is required for 17β-estradiol production by the granulosa cells, is the availability of the androgen.

Young *et al.*(1983a) demonstrated that the capacity of isolated granulosa cells of amago salmon to produce 17β-estradiol, in the presence of exogenous testosterone, increased during vitellogenesis. Young *et al.* (1983b) contend that this capacity reaches a peak at late-vitellogenesis then declines rapidly during post-vitellogenesis, coincident with the ability of oocytes in produce 17α,20β-diOHprog in response to partially purified salmon gonadotropin (SG-G100). Sakai *et al.* (1988) report that the conversion of exogenous testosterone to 17β-estradiol by follicles of medaka, increased during vitellogenesis to a level of maximal aromatase activity which occurred 20 hr before spawning. This was followed by a decrease in aromatase activity in post-vitellogenic follicles.

Kanamori *et al.* (1988) summarize the two cell model of ovarian steroid synthesis, concluding that during vitellogenesis GtH induces the thecal cells of the follicle produce androgens (mostly testosterone) which are aromatized to 17β-estradiol by the aromatase enzyme system in the granulosa layer. Immediately prior to final oocyte maturation GtH induces cells of the thecal layer to produce 17α-hydroxyprogesterone (17α-OHprog) which is subsequently transformed to 17α,20β-diOHprog by cells of the granulosa layer. At this maturational stage GtH activates the enzyme 20β-hydroxysteroid dehydrogenase (20β-HSD) which is responsible for the conversion of 17α-OHprog to 17α,20β-diOHprog. Gonadotropin receptors have been identified in preovulatory thecal and granulosa layers of amago salmon follicles (Kanamori and Nagahama, 1988a). In this study the number of receptors increased in relation to developmental changes in follicular steroidogenesis induced by GtH.

Sakai *et al.* (1987) demonstrated a shift in the steroidogenic capacity of ovarian follicles of medaka, from secretion of mostly 17β-estradiol during vitellogenesis, to secretion of 17α,20β-diOHprog during final oocyte maturation. In this study GtH stimulated production of 17β-estradiol by follicles during early vitellogenesis. Steroid synthesis by follicles changed immediately prior to and during maturation when 17α,20β-diOHprog was produced. Kanamori *et al.* (1988) report that in response to GtH, intact follicles of amago salmon only develop the potential to secrete the maturational steroid 17α,20β-diOHprog immediately prior to spawning. This investigation demonstrated that thecal layers had the capacity to produce 17α-OHprog in response to GtH prior to final oocyte maturation. Granulosa cells of late vitellogenic follicles were also shown to have the capacity to convert 17α-OHprog to 17α,20β-diOHprog indicating that the activity of 20β-HSD increased in response to GtH. These authors concluded that the pre-maturational fall in levels of 17β-estradiol observed in amago salmon was due largely to a decrease in aromatase activity in the cells of the granulosa layer and the secretion of 17α-OHprog by cells of the thecal layer in response to GtH. In tropical catfish, *Clarias macrocephalus*, the activities of the

androgen-producing enzymes 17α-hydroxylase and C-17-C-20 lyase are both diminished while the activity of 20β-HSD was increased following injection of HCG. As a result of these changed activities following HCG administration, 17α,20β-diOHprog production was stimulated while androgen production decreased dramatically (Suzuki *et al.*, 1989).

Sakai *et al.* (1989) conducted an *in vitro* study to determine the changes in steroid production by testicular fragments of amago salmon in response to chum salmon GtH, at different stages during spermatogenesis and spermiation. Results of this study show that in testes of amago salmon there is a distinct shift in the steroidogenic pathway at the onset of spermiation, from the production of 11-keto testosterone (11-ketoT) to the production of 17α,20β-diOHprog, in response to GtH. Differences in the mechanism of GtH action inducing the production of 17α,20β-diOHprog during gamete maturation was also observed between males during this study, and and that shown by female amago salmon in previous studies. The stimulatory effect of GtH on 17α,20β-diOHprog production by post-vitellogenic follicles has a 12 hour lag phase (Kanamori and Nagahama, 1988b) while the response by mature testes is immediate. It is suggested that the slower response by females is consistent with the time required for gene transcriptional and translational events. Unlike the situation in females, there is high activity of 20β-HSD in testes throughout spermatogenesis and spermiation and it is suggested that production of 17α,20β-diOHprog by males may only be limited by the availability of 17α-OHprog (Sakai *et al.*, 1989) .

A variation on the two cell model for synthesis of 17β-estradiol and 17α,20β-diOHprog is reported for *Fundulus heteroclitus* by Petrino *et al.* (1989) who conclude that the involvement of the thecal layer is not required for steroid synthesis in this species. The granulosa cells of *Fundulus heteroclitus* possess all the enzymes required for production of testosterone, 17β-estradiol, 17α-OHprog and 17α,20β-diOHprog, while cells of the thecal layer lack aromatase and secrete mostly testosterone. Such

results indicate that variations in the precise location of steroidogenic cells occur within different species of teleosts.

5.3.4 Steroid feedback.

Peter (1982) and Stacey (1984) review the work of several workers investigating the effects of gonadal steroids on the regulation of GtH release in teleosts. This information is summarised as follows.

In addition to the role of steroids in mediating the gonadal actions of GtH, ovarian and testicular steroids may act through a negative feedback interaction, to regulate the release of GtH from the pituitary. Negative feedback effects have been demonstrated by castration experiments resulting in an increase in plasma GtH following castration at spawning time. Injection or pituitary implantation of gonadal steroids reduces the high GtH levels induced by castration. The use of antiestrogen compounds (i.e. clomiphene citrate, tamoxifen) have also been shown to increase circulating levels of GtH in several species of teleosts. Autoradiographic studies have demonstrated steroid binding in the pituitary and other regions of the brain including the nucleus lateral tuberis (GnRH cell centre) and the preoptic region (GRIF cell centre). These findings suggest that fluctuations in pre-spawning levels of gonadal steroids may affect the neuroendocrine mechanisms controlling GtH release, however it is unlikely that such affects will be of significant importance.

More recently it has been suggested that the feedback regulation of GtH secretion by gonadal steroids is mediated through there intervention in the metabolism of monoamines (Manickam and Joy, 1989). DeLeeuw *et al.* (1985b) propose a mechanism for steroid- mediated negative feedback regulation of GtH release in the African catfish. In this hypothesis androgens are aromatized to estrogens and subsequently hydroxylated to catecholestrogens and catecholamines such as dopamine

in the brain and/or pituitary . Dopamine and catecholestrogens are methylated to form inactive products by the enzyme catechol-O-methyltransferase (COMT) for which catecholestrogens are better substrates. In the presence of catecholestrogens there is a decrease in methylation of dopamine resulting in its concentration and a corresponding increase in the inhibition of GnRH, consequently reducing the release of GtH. Results of investigations of the location of the enzymes involved in this proposed mechanism indicate that the gonadotrophic cells of the pituitary are possible sites of action of the gonadal steroids in the African catfish. *In vivo* studies on the catfish, *Clarias batrachus*, demonstrated that 17β-estradiol can modify the activity of the enzyme monoamine oxidase which oxidatively deaminates monoamines in the hypothalamus (Manickam and Joy, 1989).

5.3.5 Control of ovulation.

Stacey and Goetz (1982), Goetz (1983) and Stacey (1984) present good reviews of investigations into the process of ovulation in teleosts, which are summarized as follows.

Ovulation refers to the expulsion of the oocyte from the ovarian follicle. Before ovulation occurs the multitude of microvillar connections between the oocyte and the follicle must become detached (separation) to form a distinct hole in the follicle through which the oocyte passes (rupture). A possible participation by protease enzymes in follicle-oocyte separation is proposed, however oocyte rupture is believed to involve active contraction of the follicle. In medaka microfilaments are found in specific thecal cells similar to smooth muscle cells. These particular thecal cells increase in number as ovulation approaches. The microfilament contraction inhibitory compound cytochalasin B inhibits *in vitro* ovulation of medaka oocytes and partially inhibits those of trout. Results of other investigations using various methods to interfere with the contraction mechanism all suggest a necessity for muscular

contraction during ovulation in teleosts.

Investigations into the hormonal control of ovulation provide evidence for the involvement of prostaglandins (PG's) as local ovarian mediators of the ovulatory action of GtH, possibly by stimulating follicular contraction enabling rupture of the follicle and expulsion of the mature oocyte. The addition of prostaglandins (PGE_1, PGE_2, $PGF_{1\alpha}$, $PGF_{2\alpha}$) generally produces a stimulatory effect on the *in vitro* ovulation of mature oocytes from several species of teleosts, however the identity of the most potent PG varies between species which indicates that phylogenetic differences may exist. PG's are not stored and must be synthesized from specific fatty acid precursors incorporated in membrane lipids. The fatty acid precursors are mobilized from these stores by a range of stimuli (i.e. steroids, gonadotropins, mechanical agitation, hypoxia). In teleosts *in vitro* synthesis of PG's increases during the periovulatory period. Results of investigations using indomethacin (an inhibitor of prostaglandin synthesis) on goldfish indicate that PG's act at the level of the ovary, and that this action is restricted to a short time before ovulation. In goldfish the presence of intraovarian-ovulated oocytes have been shown to stimulate female spawning behavior, an effect apparently mediated by elevated levels of PG in the ovarian fluid and plasma while ovulated oocytes are present in the ovarian lumen. Studies on several species of teleosts also support a role for PG's mediating the effects of ovulated oocytes on the behavior of spawning females. It is suggested that PG's act on the brain to induce this behavior. In addition to these roles in female teleosts, PG's stimulate vasodilation and increase vascular permeability in mammals and it is suggested that in males they may mediate the effects of gonadotropin on hydration of semen.

Berndtson *et al.* (1989) have recently shown that *in vitro* ovulation of follicles from yellow perch, *Perca flavescens*, is restricted when extrafollicular tissue is removed. In addition it was shown that follicles, with tissue intact, produced significant amounts of

prostaglandin when stimulated by 17α,20β-diOHprog, whereas isolated follicular components did not. It is suspected that an intimate interaction between extrafollicular tissue and the follicle wall is necessary for synthesis of prostaglandins and subsequent ovulation, and the participation of the surface epithelium is required in this relationship. This study also demonstrated the existence of a protease in the follicular wall of yellow perch which may have a role in the degradation of collagen at follicular rupture.

5.4 Hormone induced spawning of teleosts.

In commercial fish culture spawning induction is undertaken to provide a dependable supply of fertilized eggs, for larval rearing and fry production purposes, as many of these species do not spawn spontaneously in captive conditions. Generally it is the females which fail to spawn, with oocytes completing vitellogenesis then rapidly undergoing atresia (Zohar, 1986). Some form of induced spawning needs to be undertaken to avoid this situation. Induction of spawning may be achieved by environmental manipulation. However, direct hormonal intervention is the approach investigated in this study. An understanding of the procedures used to manipulate the neuroendocrine control of reproduction in teleosts, provides the basis for research on the induced spawning of *Latris lineata*.

Summaries of the techniques used to induce the spawning of teleosts using hormones are provided by Lam (1982, 1985) and Donaldson and Hunter (1983). From the preceding description (chapter 5) it can be seen that there are a number of options for exogenous hormonal intervention in the neuroendocrine processes which control spawning of teleosts (Figure 5.1). The most common levels of exogenous hormone intervention are at the pituitary, through the administration of GnRH and GnRH analogues, and stimulation of the gonad by administration of GtH and pituitary extracts.

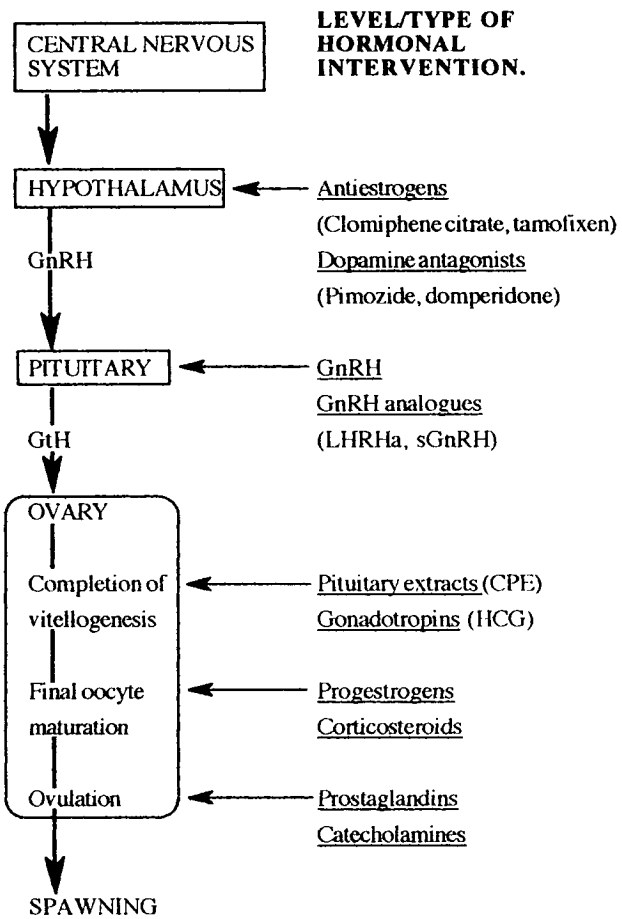


Figure 5.1 Levels of exogenous hormone intervention in the hypothalamic-pituitary-gonad axis, used to induce spawning of teleosts.
(Adapted from Donaldson and Hunter, 1983; Lam, 1985)

5.4.1 Hypophysation.

Hypophysation refers to the injection of extracts of pituitaries from sexually maturing donor fish (Lam, 1982), or the use of commercial pituitary gland preparations, such as carp pituitary extract (CPE). Although effective in inducing spawning, the standardization of this method is difficult as the amount of gonadotropin within the pituitaries used is unknown and will vary with the reproductive condition of donor fish. Problems with hypophysation also include the killing of donor fish, the possibility for disease transfer and the development of an immune reaction to the

gonadotropin when using freshly prepared aqueous extracts. Acetone dried or alcohol dehydrated, whole or powdered pituitaries can also be used. Problems also arise with the cost and supply of prepared pituitary glands (Lam, 1982, 1985; Donaldson and Hunter, 1983).

5.4.2 Gonadotropins.

The use of partially purified and purified preparations of teleost gonadotropins to directly stimulate gonadal production of steroids is an effective method of inducing spawning of captive broodstock. This method overcomes several of the problems associated with the use of pituitary glands and preparations. Partially purified gonadotropin of Pacific salmon, *Oncorhynchus* sp. (SG-100), has been used to induce spawning of a wide range of species as summarised by Donaldson and Hunter (1983), however this preparation is not cost effective when compared to crude pituitary extracts or human chorionic gonadotropin (HCG). Mammalian gonadotropins, in particular the placental hormone HCG, have been used alone or in combination with fish pituitary preparations to effectively induce ovulation in a wide range of species as reviewed by Donaldson and Hunter (1983). More recently Soletchnik *et al.* (1989) used two injections of 600 IU HCG/ kg body weight (b.w.) to induce spawning of yellowtail snapper, *Ocyurus chrysurus*. Tucker *et al.* (1991) induced spawning of Nassau grouper, *Epinephalus striatus*, using HCG at dosages ranging from 500-1000 IU/kg b.w.

Although effective several problems may arise with the use of HCG. A number of the Freshwater Commercial Fish Artificial Propagation Work Groups in China report that repeated use of HCG over consecutive years can cause fish to become resistant to it due to an immune response (Lam, 1982; Donaldson and Hunter, 1983). It is also reported that high doses of HCG may cause death by abdominal distention (Peter *et al.*, 1988). Zohar *et al.* (1989) also report that HCG induces a strong immunogenic

response following injection of gilthead seabream, *Sparus auratus*.

5.4.3 Gonadotropin releasing hormones.

In captive *Sparus auratus* the pituitary content of GtH increases approaching the spawning season however GtH levels in the plasma remains low, and although oocytes undergo vitellogenesis, they quickly become atretic. It is evident that GtH accumulating in the pituitary is not released into the circulatory system, as a result the GtH surge accompanying final oocyte maturation (section 5.3.1) and ovulation does not occur. This reproductive failure at the level of GtH release can be overcome by injection of LHRHa which induces intensive GtH secretion followed by final oocyte maturation and ovulation in female *Sparus auratus* with oocytes at, or near completion of vitellogenesis. This situation is likely to be common to many species of teleosts which do not spawn spontaneously in captivity (Zohar, 1986). The same author contends that the use of GnRH analogues confers the following advantages over hypophysation and the use of gonadotropins;

1. they stimulate release of the fishes own GtH.
2. they are easily synthesized in pure form.
3. the dosages used (µg/kg) have economic advantages.
4. they have low species specificity allowing commercially available mammalian GnRHa preparations to be used on a wide range of teleost species.
5. because they are small peptides they are not immunogenic.

Lam (1982), Donaldson and Hunter (1983) and Zohar (1986) present comprehensive reviews of the results of early research using analogues of mammalian LHRH to induce spawning in teleosts. More recently Pankhurst *et al.* (1986) showed that a single injection of HCG at 500 IU/ kg b.w. or LHRHa (des Gly^{10}[D-Ala^{6}]-LHRH ethylamide) at 100 µg/kg, followed by a second injection of LHRHa 24 h latter, effectively induced spawning in the walleye, *Stizostedion vitreum*. In this

investigation two injections of LHRHa spaced 24 h apart provided a faster response than the single injection of HCG. Garrett and Rasmussen (1987) found that for barramundi/sea bass, *Lates calcarifer*, two equipotent injections of HCG at dosages between 20-500 IU/kg b.w. administered 24 h apart caused ovulation 15 h after the second injection. Using LHRHa only a single injection of 6-25 μg/kg was required to induce ovulation on average 39 hours latter. Tamaru *et al.* (1988) investigated the minimum effective dose required to induce spawning in milkfish, *Chanos chanos* following a single intramuscular (i.m) injection of des Gly^{10}[D-Ala^{6}]-LHRH ethylamide. Although spawning could be induced with dosages between 1-5 μg LHRHa /kg b.w. these workers settled on the use of working dosages between 10-20 μg LHRHa/kg b.w. Zohar *et al.* (1989) demonstrated that spawning of female *Sparus auratus* could be induced by a single i.m. injection of 5-20 μg LHRHa/kg b.w., or 150-200 IU HCG/kg b.w. Chang *et al.* (1991) in a study of the black porgy, *Acanthopagrus schlegeli*, a protandrous hermaphrodite, showed that spermiation and oocyte maturation were stimulated during the spawning season following administration of either two i.m. injections of HCG, given 48 hours apart, at 1000 IU/ kg b.w. and 2000 IU/kg b.w., or similar injections of des Gly^{10}[D-Ala^{6}]-LHRH ethylamide at doses of 10μg/kg b.w. and 50 μg/kg b.w.

In addition to research using mammalian GnRH analogues (mGnRHa), the isolation and characterization of salmon GnRH (sGnRH) has been achieved. Analogues of this hormone have been found to be more effective in inducing ovulation in Pacific salmon than sGnRH (Donaldson and Hunter, 1983). Sherwood *et al.* (1983) determined that the structure of chum salmon GnRH was [Trp^{7}, Lue^{8}]-LHRH (tGnRH) and Sherwood *et al* (1984) have shown that this form of GnRH is found in a wide range of teleost species.

The adoption of the use of dopamine receptor antagonists in conjunction with LHRHa and sGnRH has further contributed to refinements of hormone induction methods.

The dopamine receptor antagonist pimozide has been shown to potentiate the effect of LHRHa on concentrations of serum GtH, and increase the effectiveness of LHRHa induced ovulation of goldfish (Chang and Peter, 1983). Sokolowska *et al.* (1985) showed that intraperitoneal (i.p.) injection of 10 mg pimozide/kg b.w. before injection of 100 μg LHRHa /kg b.w. increased serum GtH levels compared to fish injected with pimozide or LHRHa alone. DeLeeuw *et al.* (1985a) using the same dosages as Sokolowska *et al.* (1985), demonstrated that pimozide greatly potentiates the effect of LHRHa on GtH release and ovulation in the African catfish, *Clarias lazera*. In this study a single injection of a mixture of LHRHa and pimozide provided 100% ovulation.

Peter *et al.* (1987) demonstrated that [D-Arg^6, Trp^7, Lue^8, Pro^{9}NEt]-LHRH (sGnRH-a) in combination with pimozide, was 10 times more potent in stimulating ovulation of goldfish than [D-Ala^6, Pro^9 NEt]-LHRH (LHRH-a) in combination with pimozide. In this investigation both sGnRHa and LHRHa administered alone as a single injection, were ineffective in inducing ovulation. From investigations on a range of cultured freshwater fish in China, Peter *et al.* (1988) concluded that although injection of LHRHa or sGnRHa alone may induce ovulation, the time to ovulation was shorter and predictable when GnRH treatments where combined with a dopamine antagonist (pimozide or domperidone). In this study the combination of domperidone and sGnRHa was effective a low dosages in a number of species. In goldfish it has been found that a low dose of domperidone substantially increased the serum GtH response to sGnRHa. Domperidone is more potent than pimozide in increasing serum levels of GtH of goldfish and does not cross the blood-brain barrier in this species (and presumably that of other teleosts), which reduces the chance of detrimental effects on treated fish (Omeljaniuk *et al.*, 1987).

5.4.4 Sustained administration of GnRH analogues.

Induction of spawning of fish which have undergone completed reproductive development can usually be achieved by acute methods of hormone administration involving injection of the selected hormone. If however the intention is to initiate sexual development in juveniles or to advance the gonad development of mature fish, longer term, chronic methods for delivery of hormone/s may be necessary (Crim, 1985). Crim (1985) reviews the methods available for chronic hormone administration to fish.

5.4.4.1 Spawning induction using sustained release implants.

Huat (1980) used i.p. implanted HCG pellets to significantly increase GSI and advance gonad development of goldfish and aruan, *Ophiocephalus striatus*. LHRHa incorporated into a compacted cholesterol pellet matrix, formulated for use in mammals by Kent *et al.* (1980) has been proven to be successful for inducing spawning of teleosts. Crim *et al.* (1983a) undertook the first study using a long acting LHRHa preparation to manipulate reproduction of teleosts. In this study a single cholesterol pellet (3 x 3 mm) containing 125 µg LHRHa was implanted into the peritoneal cavity of 72 and 171 g average b.w., landlocked Atlantic salmon. Results of this study showed that plasma and pituitary GtH increased in recrudescent and prespawning female and male salmon, with pellets stimulating chronic release of GtH for up to 4 weeks.

Sokolowska *et al.* (1984) showed that injections of LHRHa (100 µg/kg b.w.) or i.p. implanted cholesterol pellets containing 25 µg and 125 µg LHRHa/fish increased circulating levels of GtH but were relatively ineffective for inducing ovulation in goldfish. Implants provided a continuous release of LHRHa causing a steady increase in serum GtH for 7-8 days depending on water temperature. No significant difference

in serum GtH profile was observed between 25 μg and 125 μg LHRHa implanted fish. However, ovulation occurred in 50% of the fish receiving the high dose pellet while no low dose implanted fish ovulated. In this study injection of 10 mg pimozide/kg b.w. 3 hours prior to, or following injection, or 18 hours following implantation, greatly potentiated GtH release response and resulted in the ovulation in 87% of treated fish.

Harvey *et al.* (1985) used i.p. implanted cholesterol pellets containing 25 μg or 50 μg LHRHa (9 - 23.5 μg LHRHa/kg. b.w.) to induce spawning of seabass, *Lates calcarifer*, and rabbitfish, *Siganus guttatus*. Almendras *et al.* (1988), investigating methods to induce spawning of *atescalcarifer*, compared the effectiveness of administering mGnRHa and sGnRHa by injection, implantation of cholesterol/cellulose pellet/s or implantation of an osmotic pump. Each 30 mg pellet was prepared with 100 μg mGnRHa or sGnRHa. The formulations of cholesterol pellet used in this study were 100% cholesterol, 95% cholesterol + 5% cellulose, and 80% cholesterol + 20% cellulose. Injection of 60 μg or 100 μg GnRHa induced a maximum of one spawning 36-48 hr after each injection. Multiple spawning was induced using continuous and pulsatile osmotic pumps, and by the implantation of an 80% cholesterol pellet together with a 95% cholesterol pellet, or implantation of two 100% cholesterol pellets. Pellets containing either mGnRHa or sGnRHa stimulated multiple spawning in this species. Seabass have also been induced to spawn using i.p. implanted pellets composed of 95% cholesterol and 5% animal lard with LHRHa combined at a ratio of 1:200 (w/w) (Garcia, 1989). Dosages used in this study ranged from 4.75μg - 300μg LHRHa/kg b.w. with an optimum dose for inducing sequential spawnings suggested to be in the range of 38-75μg LHRHa/kg b.w.

Marte *et al.* (1987) investigated the effectiveness of inducing spawning of milkfish, *Chanos chanos*, by administering mGnRHa and sGnRHa by i.m. injection (10 μg/kg), i.p. implantation of cholesterol/cellulose pellet/s (100 μg/pellet), or i.p.

implantation of an osmotic pump (330 μg/pump). Both mGnRHa and sGnRHa were effective in inducing ovulation. Cholesterol/cellulose pellet implants and injection were equally effective, inducing spawning of 75% of treated fish. Only 50% of osmotic pump implanted fish spawned. More recently Marte *et al.* (1988) report contrasting results in which milkfish i.p. implanted with cholesterol/cellulose pellets containing mGnRHa or sGnRHa (both 100 μg), showed variable responses and were less effective in inducing spawning than i.p. injection of GnRHa (24.4-33.3μg/kg) or HCG (1000 IU/kg), which were found to be equipotent.

Apart from using cholesterol based pellets, the suitability of a number of other methods of implanting GnRHa for chronic hormone release has been investigated. Pankhurst *et al.* (1986) used i.p. implanted solid silastic pellets (10 x 2 x 2 mm) containing 200μg LHRHa, to induce spawning of walleye. Pellets were prepared using Silastic 382 Medical grade Elastomer (Dow Corning Corporation) however at the LHRHa dosage used, the rate of ovulation was not increased significantly above that of control fish. Spawning of grey mullet, *Mugil cephalus*, has been achieved using implants prepared from silastic tube (20mm length x 1.92 I.D. x 0.45mm O.D.) filled with desired dosages of steroid and LHRHa dissolved in castor oil, then sealed with medical grade elastomer (Lee, 1986; Lee and Tamaru, 1988). Recently, Hirose *et al.* (1990) working with ayu, *Plecoglossus altivelis* induced ovulation of all fish implanted with a nonbiodegradable copolymer pellet containing either native LHRH (100 μg/fish) or LHRHa (50 μg/fish).

5.4.4.2 Advancement of spawning using sustained release implants.

The development of chronic hormone delivery techniques provides the opportunity to bring forward the spawning season of commercial species of teleosts to extend the period of time that eggs are available for larval rearing purposes. Such techniques can also be used to address the problem of spawning asynchrony between males and

females which occurs with captive stocks of of some species, including Atlantic salmon (Crim and Glebe, 1984) and gilthead seabream (Zohar *et al.*, 1989). Chronic hormone delivery methods are used for this application as initiation and advancement of gonad development requires the exogenous hormone to be maintained in circulation over a relatively long period of time (Crim, 1985). Previously a series of injections would be necessary to elevate and maintain levels of circulating GtH. However, the stress associated with frequent handling of broodfish may compromise the effectiveness of the hormone treatment (Billard *et al.*, 1981; Crim, 1985). Crim *et al.*, (1983b) advanced the spawning of rainbow trout by approximately 27 days using cholesterol pellets containing either 25 μg or 125 μg LHRHa i.p.implanted through the ventral body wall or via the egg pore. Spawning synchrony of Atlantic salmon was improved and the spawning occurred 4 weeks in advance of the natural spawning season when females were i.p or i.m. implanted with a 25 mg pellet containing 125 μg LHRHa (Crim and Glebe, 1984). In this species spermiation was advanced by 4 to 6 days using LHRHa delivered by silicone i.p implant (1.6 mg/kg b.w.), cholesterol pellet i.p.implant (270 μg/kg b.w.) , or by six i.p. injections of LHRHa (125 μg/kg) at two day intervals (Weil and Crim, 1983). Carolsfeld *et al.* (1988) used cholesterol/cellulose (4:1, w/w) pellets containing 100 μg mGnRHa (dose = 800-1000 μg/kg b.w.) to advance maturation of treated herring, *Clupea harengus*, by a period of at least three weeks over control fish.

Spawning of striped mullet, *Mugil cephalus*, was advanced by at least one month following i.m. implantation of 200 mg LHRHa /cholesterol pellet and 2.5 mg dissolved testosterone in a silastic capsule (Tamaru *et al.*, 1989). Barramundi/seabass have been induced to spawn one month prior to the commencement of the natural spawning season using monthly i.m. implantation cholesterol pellets containing 50μg LHRHa and 200 μg 17α-metyltestosterone (17α-MT), or 200μg LHRHa and 200 μg 17α-MT (Kuo, 1991). Although spawning has been advanced in these studies, a number of these investigations conclude that the effectiveness of the hormone

therapies increases as they approach the natural spawning season (Crim *et al.*, 1983b; Crim and Glebe, 1984; Tamaru *et al.*, 1989; Kuo, 1991).

5.4.4.3 Release of hormones from sustained release implants.

By altering the composition of the implant matrix the release rate of the hormone component can be varied providing options for the application of this technique, depending on the desired outcome. In particular, faster delivery profiles for the release of LHRHa from cholesterol pellets, can be achieved by the addition of cellulose to the pellet matrix. Sherwood *et al.* (1988) using a perfusion chamber, demonstrated that the *in vitro* release rates of GnRHa from implants varied according to their composition. All pellets contained approximately 100 μg GnRHa and were composed of 0, 25, 50, 75, 95 and 100% cholesterol, with the remainder made up with cellulose. Results showed that pellets containing between 25-100% cellulose released over 90% of the hormone within 24 h, compared to 18-20% of hormone released by 0-5% cellulose (95-100% cholesterol) pellets which only released 36-38% of the hormone over 28 days. All pellet types display a 'burst' of hormone release in the first few hours which is followed by a constant decrease in hormone release rate. It is suggested that such results are due to diffusion of the hormone through pores and channels, and by erosion or expansion of the cellulose binder on hydration.

These results are supported by an *in vivo* study using testosterone primed juvenile rainbow trout (Crim *et al.*, 1988). In this study pellets contained approximately 100 μg GnRHa and were composed of 0, 25, 50, 75, and 100% cholesterol with the remainder made up with cellulose. Plasma GtH in all treated fish remained significantly elevated up to 5 days following implantation, after which time (day 7 and 10) GtH remained elevated only in fish with implants containing >50% cholesterol. At day 10 plasma GtH was significantly higher in fish implanted with 100% cholesterol pellets, than fish treated with other pellet formulations.

5.4.4.4 Incorporation of testosterone with GnRH in sustained release implants.

Testosterone has been shown to promote pituitary GtH production in juvenile male and female rainbow trout, and it is suggested that sex steroids may influence levels of GtH in fish undergoing sexual maturation (Crim and Evans, 1979, 1983). These studies demonstrated that when either testosterone or LHRHa was administered alone, pituitary GtH increased dramatically, but plasma GtH levels remained low. However, the combination of testosterone with LHRHa was found to induce an increase in both pituitary GtH and plasma GtH levels. With respect to gonad development, GSI increased and spermatogenesis was induced in juvenile males treated with testosterone alone, or with testosterone in combination with LHRHa. Control fish and those receiving only LHRHa, showed no evidence of gonadal stimulation. Juvenile females showed no significant gonadal response to any treatment (Crim and Evans, 1983). Work conducted at the Oceanic Institute in Hawaii (Anon., 1988) established that an i.m. implanted cholesterol pellet containing 200 μg LHRHa, together with a silastic tube capsule containing 0.25 mg of dissolved 17α-MT, induced maturation of male and female milkfish. In this investigation a dose of 10 mg of 17α-MT administered alone or in combination with LHRHa produced a decrease in the circulating levels of testosterone. This result suggests that at higher dosages negative feedback inhibition by implanted 17α-MT at the level of the pituitary, may compromise the stimulatory effect of the combined hormone treatment at the level of the gonad. Tamaru et al. (1989) demonstrated that striped mullet, i.m. implanted with a 200μg LHRHa cholesterol pellet and 2.5 mg dissolved testosterone in a silastic capsule, induced accelerated oocyte development. A similar treatment using 17α-MT inhibited this development. Circulating levels of both 17β-estradiol and testosterone fell in response to the LHRHa+17α-MT treatment. These results indicate that species variability exists with respect to type of androgen used, and dosages which are stimulatory at the level of both the pituitary and the gonad.

Kuo (1991) used monthly i.m. implantation of LHRHa and 17α-MT combined in a cholesterol pellet, to significantly improve the percentage of maturation for barramundi/seabass. Maturation was induced using hormones combined in doses of 50 μg LHRHa with 200 μg 17α-MT/ kg b.w., or 200 μg LHRHa + 200 μg 17α-MT/ kg b.w. When maturation was reached spawning was induced using a single i.m. injection of 100 μg LHRHa/kg b.w. As a result of hormone implantation, oocyte development could be stimulated and maturation frequency was improved with treated fish undergoing sequential spawning and rematuration during the spawning season. Recently Trudeau *et al.*, (1993) reported that implantation of female goldfish with either testosterone or estradiol (100 μg/g in solid silastic pellets) potentiated an increase in serum GtH II in response to domperidone (10μg/g). Testosterone and estradiol also potentiated *in vitro* sGnRHa induced GtH II release from fragments of pars distalis. *In vivo* results indicate that although sex steroids enhance pituitary responsiveness to GnRH, they also increase dopamine turnover in the pituitary which increases dopamine inhibitory tone on GtH II release, thus maintaining levels of serum GtH II. These authors conclude that sex steroids play a role in modulating the relative contribution of GnRH and dopaminergic inhibitory tone, which together control the pattern of GtH II release in goldfish.

6 HORMONE INDUCTION EXPERIMENTS WITH *Latris lineata*.

Over three successive spawning seasons between 1990-1992, trials were undertaken to develop a hormone induction method suitable for inducing spawning of captive *Latris lineata* broodstock. At the University of Tasmania aquaculture facility broodstock ranging from 1.8 - 8.0 kg were maintained in a recirculating seawater system constructed for this purpose (Figure 6.1). In this system water was pumped from a 2.2m square x 0.9m deep (4.4 m^3) holding tank to a 2.0m x 0.45m x 0.35m deep (0.32 m^3) header tank. A portion of water was diverted and pumped through a venturi at the base of a 2.0m x 0.15m diameter foam fractionator, to remove dissolved organic matter. Water from the header tank flowed by gravity into a 2.0 m x 0.45m x 0.25m deep (0.25 m^3) trickling biological filter with shell-grit as the substrate, before returning to the holding tank. Salinity was monitored and adjusted to 35 ppt and pH was kept between 7.8-8.2. Water temperature was set by adjusting the airconditioner thermostat of the airconditioner which controlled the air temperature in the insulated room in which the system was housed. Photoperiod was controlled by periodic manual adjustment of lighting timers within the room.

This system placed a number of limitations on the hormone induction research which could be conducted on this species. Working on a desired stocking density of 10 kg/m^3, and an average fish weight of 3.0 kg (approx. 60.0cm F.L.), at which the majority of fish are mature (section 4.6.2.), only 14 fish could be accommodated. This limited number of fish restricted the amount of replication possible within experimental designs.

6.1 1990 Spawning season.

The objective of research in the 1990 spawning season was to trial the suitability of the commercially available hormone preparation Ovaprim ([D-Ala^6, Pro^9-NEt]-sGnRH

plus domperidone in propylene glycol solvent; Syndel Ltd.). In this investigation the dosage recommended for salmonids (0.5 ml/kg b.w.) was used. Two treatments were to be compared;

Treatment 1 : Spawning induction following the recommended injection protocol of a priming injection (25% total dosage), followed by a resolving injection (75% final dosage) 24 hr latter.

Treatment 2 : Administering the full dosage as a single injection.

Control : Injectable (normal) saline (0.5ml/kg).

On 15 August 1990, in anticipation of the coming spawning season, 8 presumed female and 6 male *Latris lineata* (1860 - 4880 g) were selected from live fish held in the well of a commercial fishing vessel at Port Arthur. At the time of collection, anaesthesia of fish to allow gonad biopsy to be conducted was not possible, because discarded fish were to be sold for consumption. Fish could only be sexed on the basis of no milt expressible = female; milt expressible = male. Fish selected were transported to Launceston in oxygenated seawater in a 2000 L fiberglass tank, transfered into the holding tank, and allowed to acclimatize for one week. Within two days fish were feeding to satiation on pieces of jack mackeral, *Trachurus declivis*, and squid. Throughout the experimental period water temperature was maintained at 12 °C with a 9: 15 L:D photoperiod.

In preparation for hormone induction, each fish was dip netted from the holding tank into an anaesthetic bath (250 L square plastic bin) prepared using 100 L of tank water with 60 ppm benzocaine added. Following suitable anaesthesia (4-5 minute induction period) fish were weighed, sexed, tagged and i.p. injected prior to being returned to the holding tank for recovery. Fish were always transfered using a vinyl cradle to avoid handling damage. During this procedure sex was determined by application of gentle abdominal pressure to induce milt flow. If milt did not flow a gonad biopsy was undertaken using a length of polyethylene post operative tubing (FG8, Indoplas Pty.

Ltd. Aust.) inserted 5 - 7cm into the genital pore (Figure 6.2). Oocytes from biopsy samples obtained were used to determine initial mean oocyte diameters for each female. Fish were tagged with an anchor tag, to which a 5-7 mm section of coloured tubing was attached, so that individual fish could be visually identified in the holding tank. The hormone treatment was injected into the mid-ventral peritoneal cavity, approximately 5 cm posterior to the pelvic fin, using a 1ml or 2ml syringe with a 21 guage x 38mm needle.

At the time of the first injection, biopsy samples showed that two females had only primary stage oocytes present, and were assessed as being immature. No biopsy sample could be obtained from another two fish which were assumed to be immature males. These findings reduced the number of usable female fish from 8 to 4. It was decided that the 4 suitable females should be used to assess the the recommended dosage of Ovaprim, as this product had not previously been trialed on this species. Of the 4 females, 3 were injected following the protocol recommended for Ovaprim (treatment 1) and the remaining female was treated as a control.

Oocyte samples were taken at the time of the first injection (0 hrs), following the onset of abdominal swelling (+72 hrs) and again 72 hours latter (+144 hrs). No oocyte sample was taken when the second hormone injection (+24 hrs) was administered. Oocyte samples were measured immediately using a binocular microscope with an ocular micrometer, and mean oocyte diameter was determined for each fish (Table 6.1).

Samples taken at +72 hrs and +144 hrs showed two distinct batches of oocytes, an ovulated first batch and a second batch developed to late vitellogenesis. These two batches of oocytes are separated in the presentation of the results from this investigation (Figure 6.3).

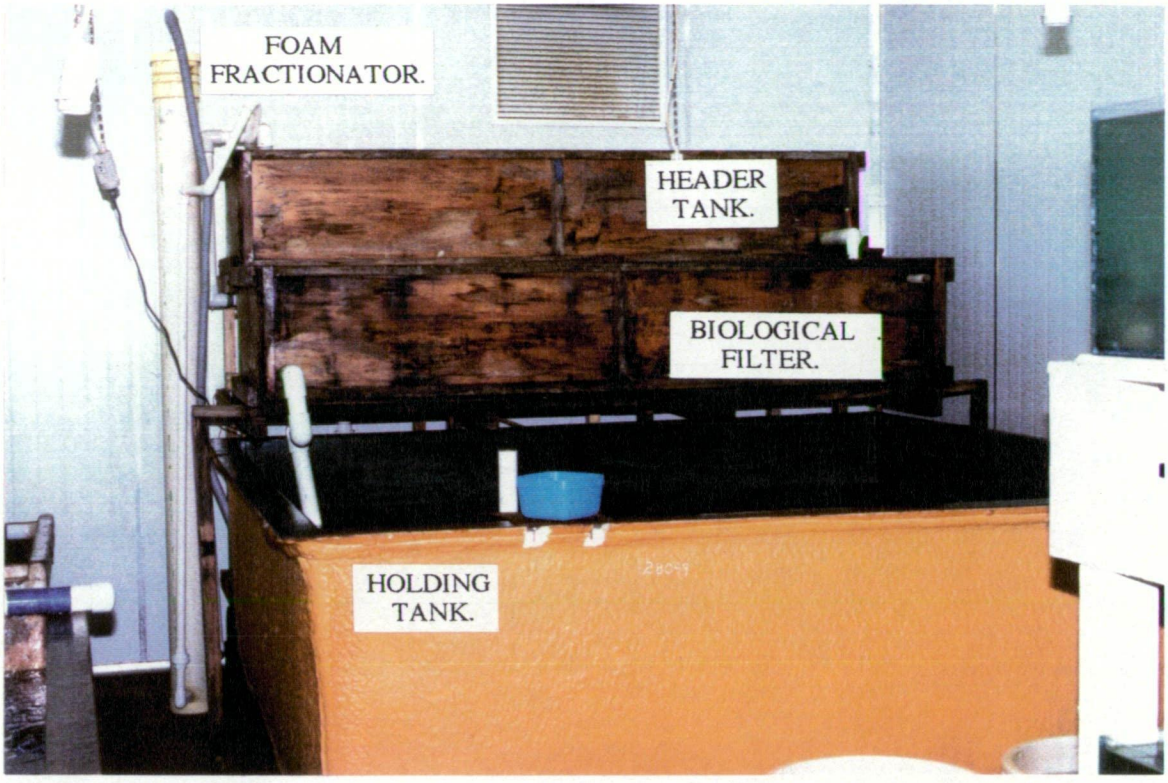


Figure 6.1 Recirculating seawater holding system used to hold *Latris lineata* broodstock for hormone induction research.

Figure 6.2 Biopsy procedure being conducted on female *Latris lineata* using polyethylene tubing inserted into the ovary via the genital pore.

Table 6.1 Mean diameter of batches of oocytes from Ovaprim treated fish (n=3) and control fish (n=1) following i.p.injection of *Latris lineata*.

(S.E.= Standard Error)

OVAPRIM	HOURS POST INJECTION/ OOCYTE BATCH MEAN DIAMETER (um) +/- S.E.									
TREATED	0		72				144			
FISH NO.	First	S.E.	First	S.E.	Second	S.E.	First	S.E.	Second	S.E.
1	823.8	11.3	1195.9	7.3	722.2	11.1	1248.2	8.2	741.8	11.7
2	766.6	10.9	1175.5	8.9	756.5	15.7	1184.6	8.9	-	-
3	689.0	8.1	1184.5	7.9	728.1	18.2	-	-	746.7	9.7
TREAT. MEAN +/- S.E.	760.0	39.1	1185.3	10.1	735.3	10.5	1216.5	31.5	744.5	2.5
CONTROL	683.3	10.5	711.9	9.0	-	-	707.1	7.4	-	-

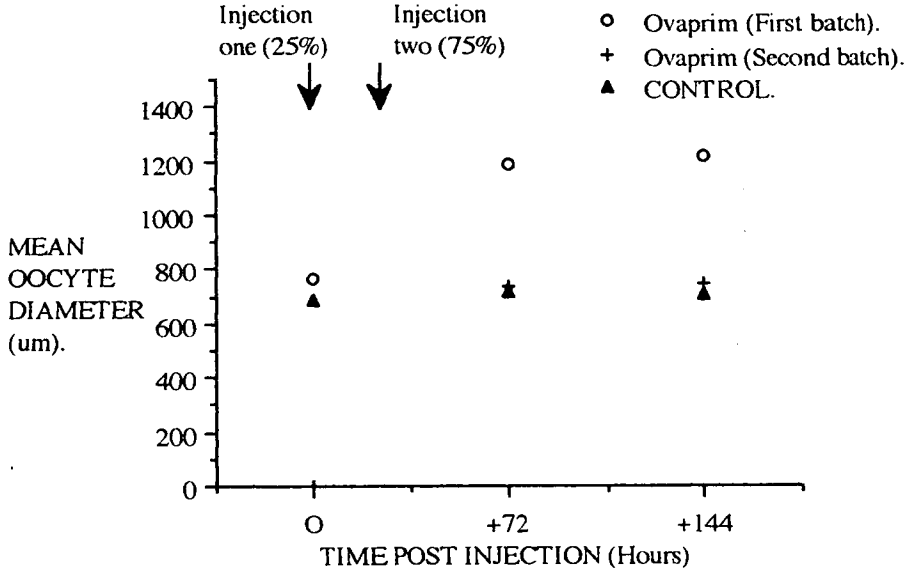


Figure 6.3 Mean diameter of batches of oocytes from Ovaprim treated (n=3) and control (n=1) *Latris lineata*.

These results suggest that Ovaprim could be used to induce spawning of *Latris lineata* however manual stripping of ovulated eggs may be necessary, as no spontaneous spawning was observed. The chance exists that spontaneous spawning may have

occurred within the tank. However, no eggs were found on the water surface or within the water column during sampling using a fine mesh dip net. From the results presented (Figure 6.3), it is suggested that in response to hormone treatment the leading batch of oocytes was induced to ovulate however the hydrated eggs were retained within the ovarian lumen.

The possibility of inducing multiple spawnings also exists as two distinct batches of pre-spawning oocytes could be distinguished within biopsy samples taken. These results indicate that following final maturation of the leading (first) batch of oocytes, a second batch of oocytes was recruited and quickly completed vitellogenesis. This is in agreement with the conclusion presented in section 4.5, that the pattern of reproductive development in *Latris lineata* is group-synchronous, with individual fish spawning a number of times during the spawning season. Larger numbers of mature females would be required to establish this conclusively.

Such numbers would also be required to undertake meaningful investigations to establish the most effective Ovaprim dosage and the optimal timing for manual stripping of *Latris lineata*. All eggs stripped from females in this investigation did not fertilize, indicating that eggs had been retained too long in the ovary and had become over-ripe (Kjesbu, 1989). Oocyte samples showed that hydrated, ovulated eggs retained within the ovarian cavity progressively collapsed and lost their spherical shape. Ovulated eggs remain viable for only a limited time within which stripping and fertilization must occur (Hirose *et al.*, 1979). The time taken for over-ripening of eggs to occur following ovulation varies with species. For turbot, *Scophthalmus maximus*, over-ripening occurs after 10 hours while the process commences after 1-2 hours in halibut, *Hippoglossus hippoglossus*. In striped bass, many carp species and other cyprinids, over-ripening of ovulated eggs may begin less than 60 minutes following ovulation (Zanuy, 1990). Eggs of Japanese flounder, *Limanda yokohamae*, remained viable for 2 -3 days following ovulation depending on the type of GtH used to induce

ovulation (Hirose *et al.*, 1979). Eggs of *Procchilodus platensis* remain viable for less than 2 hr such that the hatching percentage of eggs stripped and fertilized after 3 hours was nil (Fortuny *et al.*, 1988)

In order to avoid over-ripening, the time at which ovulation occurs following hormone induction must be determined for *Latris lineata*, as has been deduced in other species of teleosts. Oocytes of Nassau grouper females ovulate between 48-51 hours after the first of two i.m. injections of HCG (700 IU/kg b.w.) administered 24 hr apart (Tucker *et al.*, 1991). Milkfish are reported to be ready for stripping 10 hours after the second of two i.m. injections of GtH (HCG and acetone dried chum salmon pituitary gland homogenate) spaced 9-12 hours apart (Juario *et al.*, 1984). Ovulation occurs in barramundi/seabass 39 hours after a single i.m. injection of LHRHa or the first of two injections of HCG (Garrett and Rasmussen, 1987). Fortuny *et al.* (1988) followed sampled oocytes of Sabalo, *Procchilodus platensis* at 30 minute intervals following hormone induction (homologous pituitary extract, 1.0/kg) in fish maintained at different water temperatures between 24-28 °C. This investigation demonstrated an inverse linear relationship between water temperature and the latency period between injection and ovulation. The latency period ranged from approximately 9 hr at 28 °C to approximately 12.5 hr at 24 °C.

The results from this investigation of induced spawning of *Latris lineata*, suggest that Ovaprim induces ovulation in this species. Ovulation occurs within the first 72 hr interval following the first injection (25% total dose, 0 hr), and most likely within the 48 hr interval after the second injection (75% total dose at +24 hr). Oocytes retained in the ovarian cavity become over-ripe following ovulation.

6.2 1991 Spawning season.

Approaching the 1991 spawning season the Tasmanian Department of Sea Fisheries (DSF) made available 24 *Latris lineata* broodstock, and holding facilities at the DSF Taroona laboratories (Hobart) to enable further hormone induction investigations to be conducted. The DSF wished to establish the effectiveness, and optimum dosage of LHRHa ([D-Ala^6, des Gly^{10}] mGnRH; Syndel Ltd.) for inducing spawning of *Latris lineata*. To verify results of 1990 research the recommended dosage of Ovaprim was included in this trial, which consisted of the following treatments, each replicated with 3 females;

Treatment 1.	Ovaprim	0.5 ml/kg b.w.
Treatment 2.	LHRHa	10 µg/kg b.w.
Treatment 3.	LHRHa	25 µg/kg b.w.
Treatment 4.	LHRHa	50 µg/kg b.w.
Control.	Saline	0.25 ml/kg b.w.

Broodstock used in this trial came from a stock of DSF broodfish held in a net cage (5m x 5m x 5m) at the Aquatass Pty. Ltd. marine farming lease located in North West bay near Howden, south of Hobart. These fish had been captured from the wild, tagged and kept at this location since the previous (1990) spawning season. Selection of fish for this investigation was conducted on 12 September 1991. Cage netting was gathered up to confine the fish, which were dip netted into an anaesthetic bath (40 ppm benzocaine) and examined for gonad development. 15 females and 9 males were selected for use in the trail based on the following criteria;

Males - presence of free running milt

Females - oocyte development at an advanced stage as determined by ovarian biopsy.

An ovarian biopsy was conducted on each female using an endometrial biopsy device (Laboratoire CCD, Paris) inserted into the ovary via the genital pore. Ovarian biopsy samples showed that few females had completed vitellogenesis. The best available fish were selected by visual assessment of oocyte samples which were then transfered into individually labelled 50 ml containers with 20 ml of 4% formalin in phosphate buffered saline (4% FPBS). Tag numbers were recorded and each fish was weighed prior to transfer into oxygenated seawater in a transport tank for recovery. Selected fish were transported to DSF Hobart/Taroona laboratories were 3 males and 5 females were randomly stocked into 3 x $4m^3$ square fiberglass tanks (total of 8 fish/tank). Tanks were located indoors, receiving diffused natural lighting and flow through ambient temperature seawater (10 °C) pumped from the adjacent D'Entrecasteaux channel. Fish were fed to satiation every second day during this trial with salmon growout pellets (Gibsons Ltd.). The hormone induction investigation started the day following transport of fish to the Taroona laboratories.

Measurement of initial samples taken prior to hormone treatment, showed a wide range of oocyte diameters existed between the developing females selected for this investigation. Consequently a decision was made to divide fish into 3 size categories based on the mean diameter of initial oocyte samples;

Oocyte size category	Oocyte size range (µm)	Mean oocyte diameter	+/- Standard deviation.
SMALL	360 - 442	408.8	32.1
MEDIUM	454 - 492	473.6	17.2
LARGE	538 - 804	630.8	104.5

One fish from each of these categories was randomly assigned to each treatment, so that all treatments contained fish with a similar range of initial mean oocyte diameters.

In view of the findings of the 1990 investigation the sampling interval used in 1991 was shortened to 48 hours in an attempt to better identify the time of ovulation. To reduce handling stress hormone treatments were administered at these sampling times thus the time between the first and second hormone injection was increased to +48 hr, as opposed to +24 hr employed in the 1990 trial. Injections were all given between 900 - 1100 hours and were administered in the same way as described for the 1990 trial (section 6.1). As in the 1990 trial, 25% of the total treatment dosage was injected as a priming injection (0 hr) with the remaining 75% given given in a second injection (+48 hr). The oocyte sample taken during fish selection the previous day was used as the initial oocyte sample, rather than take another sample at the time of the first injection. Because of the poor initial state of oocyte development in the majority of fish used, a third injection of 100% hormone dosage was administered to all fish at +96 hr. Hence the sampling/injection protocol used in this investigation was;

Day 1	(0 hr.)	Priming injection	25%	total dosage
Day 3	(+48 hr.)	Resolving dose 1.	75%	total dosage
Day 5	(+96 hr.)	Resolving dose 2.	100%	total dosage
Day 7	(+144 hr.)	Final oocyte sample		

Oocyte samples were preserved in 4% FPBS and measured the following day. From each sample 20 of the largest oocytes were measured to the nearest 20 μm using a Nikon profile projector. The largest 10 oocytes measured were used to determine the mean oocyte diameter for each sample (+/- standard error, S.E.).

Mean oocyte diameters obtained from individual *Latris lineata* sampled during this investigation is presented in Appendix 17. Change in mean oocyte diameter for each hormone treatment, over the 144 hr sampling period, is presented in Figure 6.4. The F_{-max} test (Sokal and Rohlf, 1973) was used to confirm that the data was

homogeneous. Two-factor repeated measure ANOVA (Appendix 18a) of the 48 hourly changes in mean oocyte diameters of injected fish reveals that none of the hormone treatments had any significant effect ($P > 0.05$) on mean oocyte diameter, at any sampling time. In this analysis an almost significant ($P = 0.051$) effect was attributed to sampling time, indicating that at different 48 hr intervals a treatment effect may have been present. Consequent one way ANOVA followed by comparison of means (Appendix 18b), at each 48 hr sampling interval following the initial (0 hr) injection, showed that there was no significant difference ($P > 0.05$) between the mean oocyte diameters of any treatment, at any of the sampling times. Further one way ANOVA (Appendix 19) of the total change in mean oocyte diameter over the 144 hr sampling period (mean O.D. at 144 hr - mean O.D. at 0 hr) also showed that there was no significant effect ($P > 0.05$) of any of the injection treatments used in this investigation.

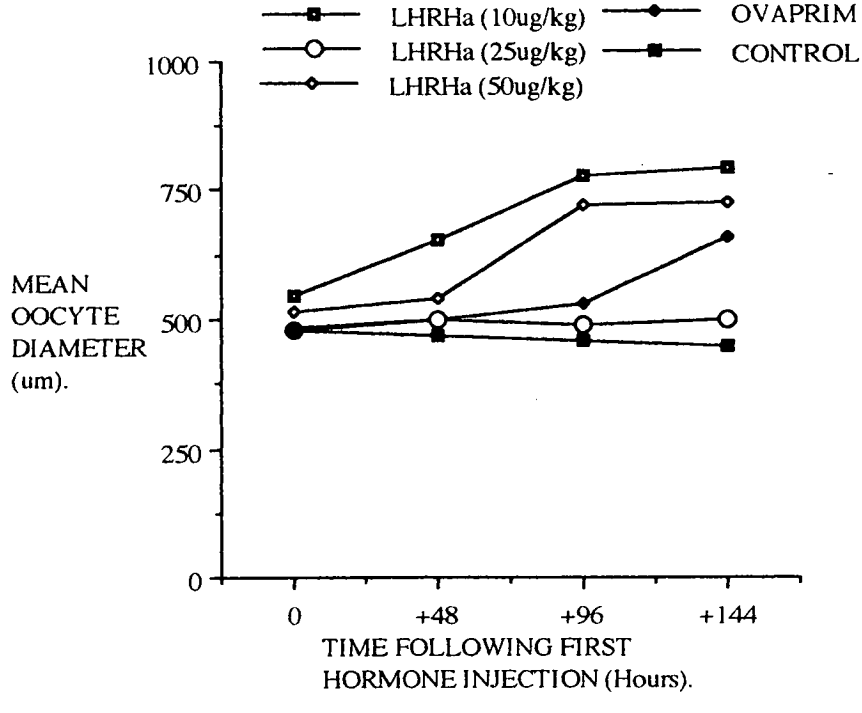


Figure 6.4 Change in mean oocyte diameter (μm) in *Latris lineata* following hormone treatment to induce spawning. (All points represent the mean of 3 samples.)

No significant ($P>0.05$) effect for any hormone treatment.

Following these findings, injection treatment was ignored and a two-factor repeated measures ANOVA was conducted on the change in mean oocyte diameters of fish with different initial oocytes size categories (Appendix 20 a). Each injection treatment was represented equally (N = 1) within each size category. This analysis revealed that the initial oocyte size category had a significant effect ($P< 0.05$) over the 144 hr sampling period. The repeated measure (48 hr sampling interval) was also found to have a significant effect ($P< 0.05$) as did the interaction ($P< 0.05$) between initial oocyte size category and sampling interval. These results indicate that the change in mean oocyte diameter does not show a uniform trend among the three size categories.

One way ANOVA of the total change in mean oocyte diameter over the 144 hr sampling period shows a significance effect ($P < 0.05$) due to initial oocyte size category (Appendix 20b). Comparison of means establishes that the total change in mean oocyte diameter for fish with large oocytes initially was significantly greater ($P< 0.05$) than those with small oocytes initially. No significant ($P> 0.05$) total change in mean oocyte diameter was recorded between fish categorized as having small and medium size oocytes initially, or between fish with large and medium initial oocyte size categories.

None of the fish used in this study were known to have spawned. Although a small number of ovulated, hydrated eggs could be manually stripped from two fish at the +96 hr and +144 hr sampling. In both cases eggs were not free flowing with a reasonable amount of abdominal pressure.

Using fish with large initial oocyte diameters (one fish only per treatment) an insight into the time to ovulation of hormone treated *Latris lineata* can be gained. In section 4.3.1.4 results of histological sampling suggested that mature oocytes attained a diameter approaching 900 μm at ovulation This observation is supported

in section 4.5, where females classified as being mature, have oocytes with a mean diameter of 849.4 +/- 30.1μm. On the basis of this information results presented in Figure 6.5 suggests that at a water temperature of 10 °C ovulation occurs within the 48 hr period following the second (75% total dose) injection of LHRHa (n=2). The Ovaprim treated fish represented, required a third injection (100% total dose) to induce ovulation in the following 48 hr period.

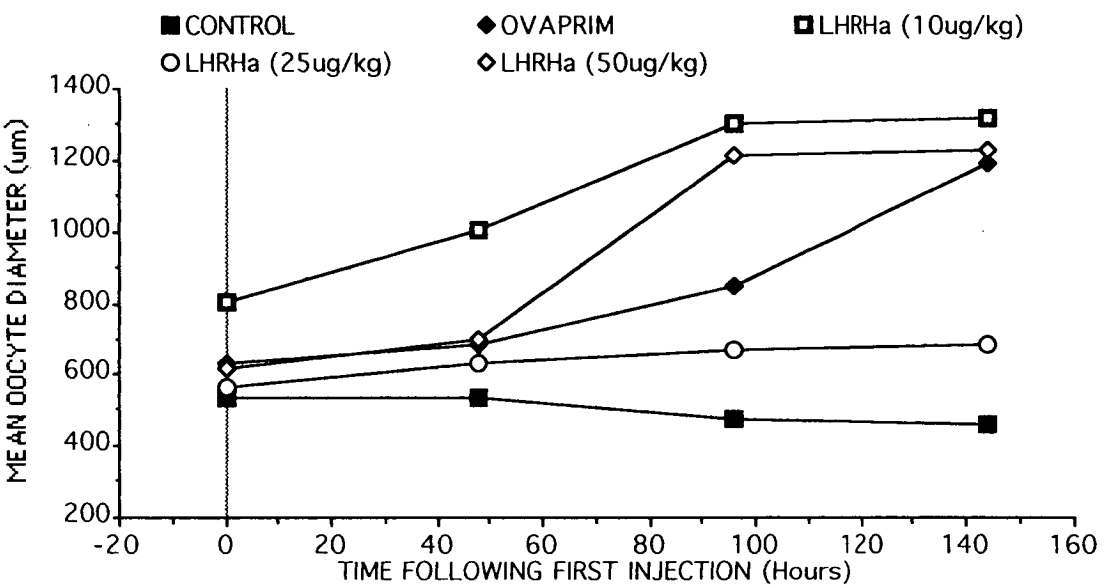


Figure 6.5 Change in mean oocyte diameter (μm) over 144 hours in response to hormone treatment of individual *Latris lineata* with large category oocytes.

In the 1991 DSF investigation 3 of the 12 hormone treated fish matured oocytes in response to hormone induction. The effect of initial oocyte size on the response of *Latris lineata* to spawning induction is demonstrated graphically in Figure 6.6 Of interest is the observation that all fish in this large category had initial mean oocyte diameters in excess of 600 μm (614 - 804 μm). These figures are in agreement with the mean oocyte diameters for fish classified as being at the late vitellogenic (605.3 +/- 36.6 μm) and migratory nucleus (622.1 +/- 38.0 μm) stages of development in section 4.5. Also worthy of note is the observation that the fish

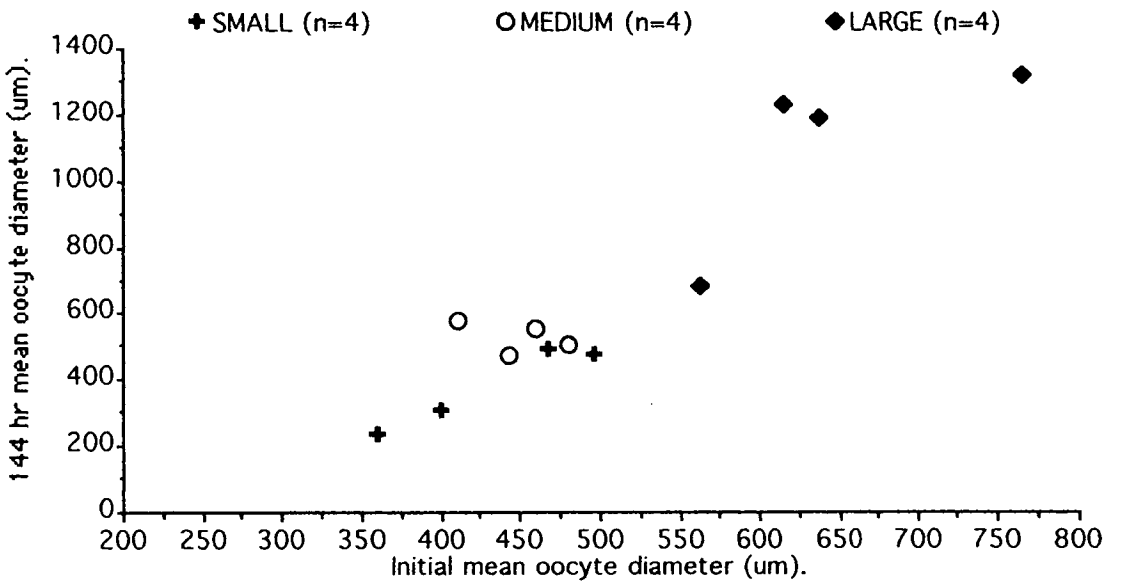


Figure 6.6 Change in mean oocyte diameter (μm) after 144 hr of hormone treatment for *Latris lineata* with different initial oocyte size categories.

with the largest initial mean oocyte diameter (804 +/- 10.2 μg) responded to the lowest dosage of LHRHa (10 μg/kg b.w.). These results must be treated as being purely descriptive rather than experimentally valid, however they stress the need to initiate hormone induction with a group of females which are uniformly advanced with respect to oocyte development, preferably with oocytes in excess of 600 μm.

Initial oocyte diameter has a great effect on the success which can be achieved from hormone induced ovulation and spawning. Kuo *et al.* (1974) suggest that hypophysation of *Mugil cephalus* is best achieved using females with an initial mean oocyte diameter of at least 600 μm, and preferably larger than 650 μm. In this study an inverse relationship was found between initial oocyte diameter and the dose of GtH required to induce spawning. A similar situation is reported for rabbitfish were fish with oocytes 0.47 mm in diameter can be induced to spawn using HCG. However, a higher dose of HCG is required if oocytes are less than this diameter (Juario *et al.*, 1985). Female barramundi, *Lates calcarifer*, with

oocytes larger than 0.4 mm are used for hormone induced spawning. Oocytes less than 0.4 mm are regarded as still maturing and respond poorly to hormone treatment (Garrett and Rasmussen, 1987). In blue-spotted grouper, *Epinephelus fario*, spawning can be induced with two daily injections of 1000 IU/kg HCG when mean oocyte diameter exceeds 0.55 mm. If mean oocyte diameter is less than 0.55 mm a third injection is required (Kuo *et al.*, 1988)

In milkfish, females classified as being at tertiary yolk globule stage (oocytes filled with yolk; Yamamoto, 1956), with initial mean oocyte diameters less than 0.66 mm did not respond satisfactorily to HCG or salmon pituitary homogenate (Juario *et al.* (1984). Marte *et al.* (1987) suggest that differences in initial oocyte diameters of milkfish, reflected different degrees of completion of vitellogenesis, which contributed to the variable responses found for hormone induction using injections, implants and osmotic pumps. From another investigation of milkfish Tamaru *et al.* (1988) conclude that the stage of development at which fish are most receptive to spawning induction using LHRHa is characterized by a unimodal size distribution of oocytes averaging 750 μm. Milkfish undergo a series of group synchronous oocyte maturations (as shown to be the pattern in *Latrislineata*), developing uniform clutches of large oocytes from a more variable clutch of smaller oocytes. This investigation suggests that, as in other teleosts, there is a high correlation between stage of oocyte maturation and circulating levels of 17β-estradiol and testosterone. These authors observe that spawning was not induced using LHRHa when oocyte diameters were between 600-700 μm, the time at which highest levels of 17β-estradiol was recorded, which is consistent with the effects of a steroidal negative feedback mechanism operating at this stage. The level of 17β-estradiol declined markedly at the end of vitellogenesis (oocyte diameter > 750 μm) while the level of testosterone increased. This pattern is in agreement with a decrease in the negative feedback mechanism and supports the notion of testosterone serving to stimulate GtH synthesis by the pituitary, prior to

the pre-ovulatory GtH surge.

The results of the 1991 DSF hormone induction trial have not demonstrated the effectiveness of any of the hormone treatments used as no eggs were stripped from any fish. There is no indication that spontaneous spawning occurred although this is a possibility in the flow through system used. The latency period between hormone treatment and ovulation has not been clearly demonstrated in this study. The limited and variable state of oocyte development of females at the time of commencement of this trial has undoubtedly contributed to these outcomes. Results suggest that *Latris lineata* will be more responsive to hormone induction procedures if fish used possess a uniform batch of oocytes with initial mean oocyte diameters exceeding 600 μm. This is in agreement with mean diameters of oocytes classified as being at vitellogenic and migratory nucleus stages of oocyte development (section 4.5) for this species.

6.3 1992 Spawning season.

Results of 1990 and 1991 investigations suggested that the aim for the 1992 spawning season should be to induce uniform reproductive development of *Latris lineata*. In order to achieve this objective it was decided to investigate the use of sustained release implants described in section 5.4.4. In conjunction with the use of implants the possibility to conduct a limited number of serum steroid assays became available to document some of the endocrinological responses of fish treated with sustained release hormone implants.

Approaching the 1992 spawning season two complimentary investigations were undertaken, one at the DSF Taroona laboratories and the second at the University of Tasmania at Launceston (UTL), Key Centre for Aquaculture. Both of these experiments primarily aimed to assess the efectiveness of a series of monthly i.m.

implanted sustained release cholesterol pellets containing a combination of LHRHa and 17α-methyltestosterone (17α-MT) to promote uniform gonad development in *Latris lineata* broodstock. Cholesterol pellets (90% cholesterol + 10% solidified coconut oil/copha binder) were prepared with dosages of LHRHa and 17α-MT based on those which had proven to be successful for inducing oocyte maturation and spawning of milkfish (Tamaru, 1988) and barramundi (Kuo, 1991). In both the DSF and the UTL experiments, a series of three monthly i.m. implants were administered. In the DSF experiment a high dose pellet was compared with a low dose pellet (50% of high dosage) formulation. At the UTL only the high dose pellet formulation was used. In both investigations control fish were implanted with 'placebo' pellets prepared without the hormones. Hormone dosages of pellet implants used in these experiments were as follows;

Implant			Pellet formulation.	
number	Month	Hormone	HIGH(DSF and UTL)	LOW (DSF only)
1.	July	LHRHa	50 μg	25 μg
		17α-MT	200 μg	100 μg
2.	August	LHRHa	200 μg	100 μg
		17α-MT	200 μg	100 μg
3.	September	LHRHa	200 μg	100 μg
		17α-MT	200 μg	100 μg

In order to follow changes in the levels of circulating steroids after implantation of sustained release hormone pellets, a series of blood samples were taken from treated fish. In the DSF experiment blood was sampled from all fish at the time of implantation (0 hr) and at approximately the same time, the following day (+24hr). Due to the limited funds available to finance steroid assays blood samples were taken from only half of the fish in each treatment at +24 hr. In the UTL investigation blood samples were taken from all fish at the time of implantation,

and from 3 hormone implanted females, at 2 day intervals (+48hr, + 96 hr) until day 6 post-implantation (+144 hr), at which time blood samples were taken from control fish and males also. The blood sampling protocol for both experiments was thus as follows;

	Blood sampling time (hours)				
Experiment.	0	+24	+48	+96	+144
DSF	*	*	-	-	-
UTL	*	-	*	*	*

* = Blood sample taken.

6.3.1 Pellet preparation procedure.

Pellet formulation and preparation were based on descriptions given by Lee *et al.* (1985, 1986) although a number of modifications were made to this procedure. The total quantity of LHRHa ([des-Gly^{10}, D-Ala^{6}, Pro^{9} ethylamide]-LHRHa) required for both investigations was calculated based on hormone dosages and an average fish weight 3.0 kg. This total amount of LHRHa (63 mg) was supplied as single measured quantity from the manufacturer (Peptide Technology Ltd. Sydney). For ease of pellet calculations a pellet formulation which provided a final relationship of 10.0 mg pellet : 1.0 kg b.w. was adopted. Rather than dissolving LHRHa in ethanol and drying at 30-35 °C (Lee *et al.*, 1985,1986) a dry mixing procedure was used following advice that dissolving and drying may reduce the efficacy of the hormone (Pers. Comm. Mr. Brian Day UTL).

A pellet mould for 24 x 6.0 mm x 2.0 mm diameter pellets was constructed (Figure 6.7) following the directions of Lee *et al.* (1985,1986). Preliminary attempts at pellet manufacture revealed the need to ensure that the template and base plate of the mould be securely fixed using 6 closely spaced bolts, to avoid

loss of pellet material into the interface between the two plates during pressing. Difficulties in removing pellets were encountered with early mould prototypes constructed of 6.0.mm clear perspex sheet. Removal of pellets from the mould necessitated hammering them out using a hole punch, which caused pellets to bend and fracture. This problem was overcome by constructing templates from 6.0 mm polyethylene sheet which has superior slippage characteristics which enabled pressed pellets to be easily pushed from the mould with little or no damage.

In the course of early trials it was found that 10% copha binder needed to be incorporated with the cholesterol powder, to provide good pellet formation and stability during handling. Preliminary trials were conducted to find a way to accurately and simply dispense molten copha. A small amount of copha was placed in a small beaker suspended in a larger beaker of hot tap water. When molten, the copha was drawn into a 1 ml syringe fitted with a rubber pipette bulb. A 25G needle was fitted and 5 drops of copha were expressed onto a glass slide which was then and transfered onto a tared Mettler AE 210 delta range electronic balance (0.0000g), and the weight recorded. During this procedure the needle must be held near vertical, with inclined flat surface facing down to allow immediate release of uniform size drops. This process was repeated 10 times providing a mean weight of 20.6 +/- 1.3 mg per 5 drops, or approximately 4.0 mg/drop, which was considered suitable for the requirements of this investigation.

To assist weighing accuracy, stock hormone powders (1:20 w/w) were made up for LHRHa (63 mg) and 17α-MT (100 mg; Sigma Chemical), both in combination with cholesterol (BDH Chemicals). Each stock hormone was ground with a small mortar and pestel for 10 minutes to mix thoroughly, transfered into a storage container, sealed and refrigerated until use. Pellets were prepared in batches of 500 mg, sufficient to implant 50 kg of broodstock. Constituents of all pellets used are presented in Appendix 21. Control pellets were made in the same manner without

either hormone (90%cholesterol + 10% copha).

All dry ingredients were weighed out seperately on the electronic balance described then mixed thoroughly for 5 minutes by folding together using a microscope slide on a flat glass plate positioned over a warm water bath sufficient to maintain coconut oil (melting point 21-25 °C) in a molten state during mixing. The required number of drops of molten copha were distributed over the powder and mixed by folding and cutting for a further 5-10 minutes. The crumby mix was then transfered onto the pellet mould and packed sequentially into the holes using a 2.0mm hole punch. During this procedure the glass slide was used to distribute the dry mix and a small brush used to retrieve any remaining mixture from surfaces. Initial trials of the complete pellet manufacture procedure returned loss rates of 16.7% and 16.6%, however this loss was reduced to 10.2 - 12.4% by the time of pellet preparation for the implantation trials.

Following removal from the mould each pellet was weighed. The length of each pellets from the first three batches was measured to the nearest 0.05mm and the pellet density determined;

Batch No.	Mean length (mm)	+/- S.D.	Mean weight (mg)	+/- S.D.	Mean density (mg/mm)
1.	5.80	0.20	18.87	0.82	3.25
2.	5.63	0.15	17.94	0.75	3.19
3.	5.42	0.47	17.88	2.07	3.30

In preparation for use each pellet was weighed and trimmed with a scalpel to the nearest milligram (+/- 0.1mg) and stored in a sealed, labelled 3ml tube with other pellets of the same weight, thus providing a range of pellet sizes.

6.3.2 Pellet implantation and blood sampling.

A simple implantation device consisting of a thin walled 2.5 mm I.D. stainless steel tube, cut diagonally and sharpened at the insertion end, was used to implant pellets into fish. The implanting tube was fitted into a slightly undersize hole in a wooden handle with a large diameter conical hole feeding into the tube from the opposite side which enabled the loading of pellets. A wire rod was used to push pellets to the base of the implanter as can be seen in Figure 6.8.

In both investigations fish were anaesthetized in an aerated seawater bath using 60 ppm benzocaine. Fish were weighed to the nearest 100 g and a combination of pellets selected appropriate for a fish of that weight. A scale was lifted and a 3-4mm incision was made with a No. 11 scalpel blade, 3 - 4 cm below the first dorsal spine, into the anterior dorso-lateral muculature. The implanter was inserted 2-4 cm into the muscle, or until until the skeletal frame was felt (Figure 6.8). Selected pellets were loaded into the handle of the implanter and pushed to the base with the wire rod, the implanter was then twisted and gently withdrawn. The scalpel and implanter were transfered into alcohol between fish to disinfect. Figures 6.9 and 6.10 show respectively the healed point of implantation, and the position of the pellets found within the anterior dorso-lateral musculature of an implanted fish which died two months after these investigations.

At the time of implantation a gonad biopsy was conducted and oocyte samples were preserved in 4% FPBS, as described previously. Each fish was tagged and weighed and a blood sample was taken from the caudal vien using a 21G x 38mm needle and 2.5 ml Terumo syringe. At least 2.0 ml of blood was collected and transfered into a 3 ml siliconized plastic tube which was then capped and placed in a container surrounded with flake ice to clot. A liberal coating of betadine antiseptic ointment (Faulding Pharmaceuticals, Adelaide) was applied to the

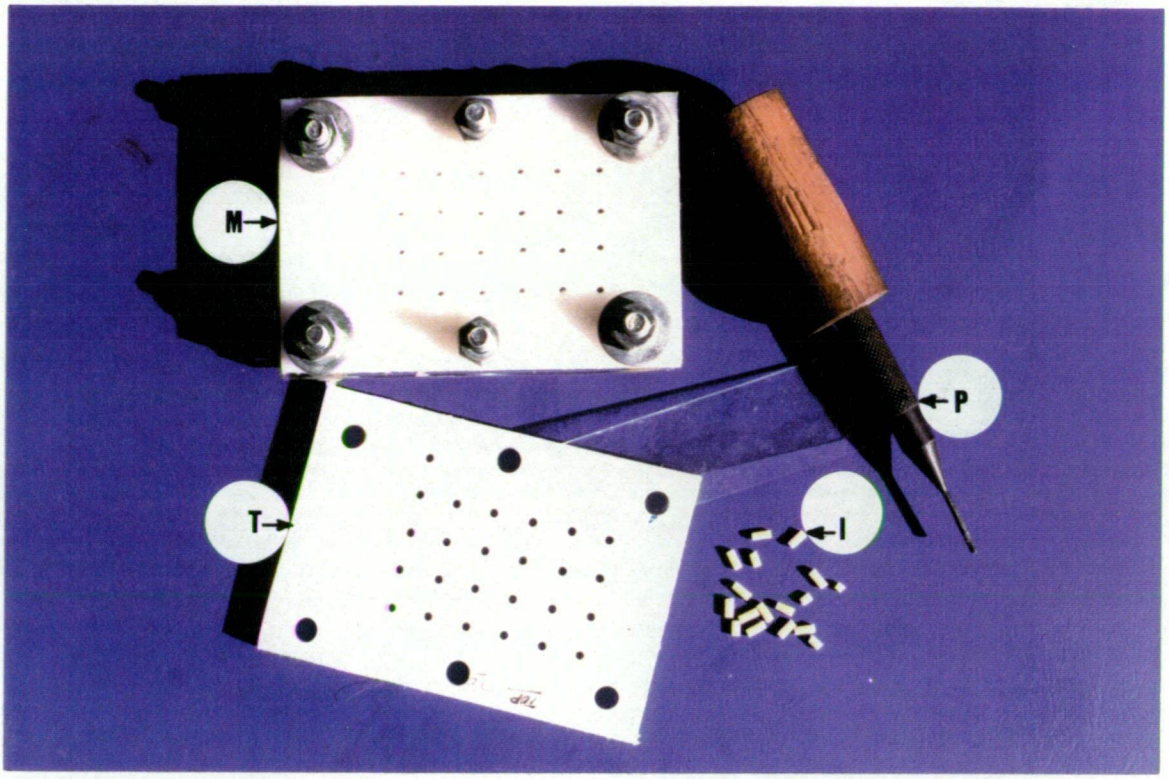


Figure 6.7 Equipment used to make sustained release hormone implants/pellets. (M = assembled pellet moult; T = template for larger diameter pellets; P= hole punch for packing and removal of pellets; I = finished pellets)

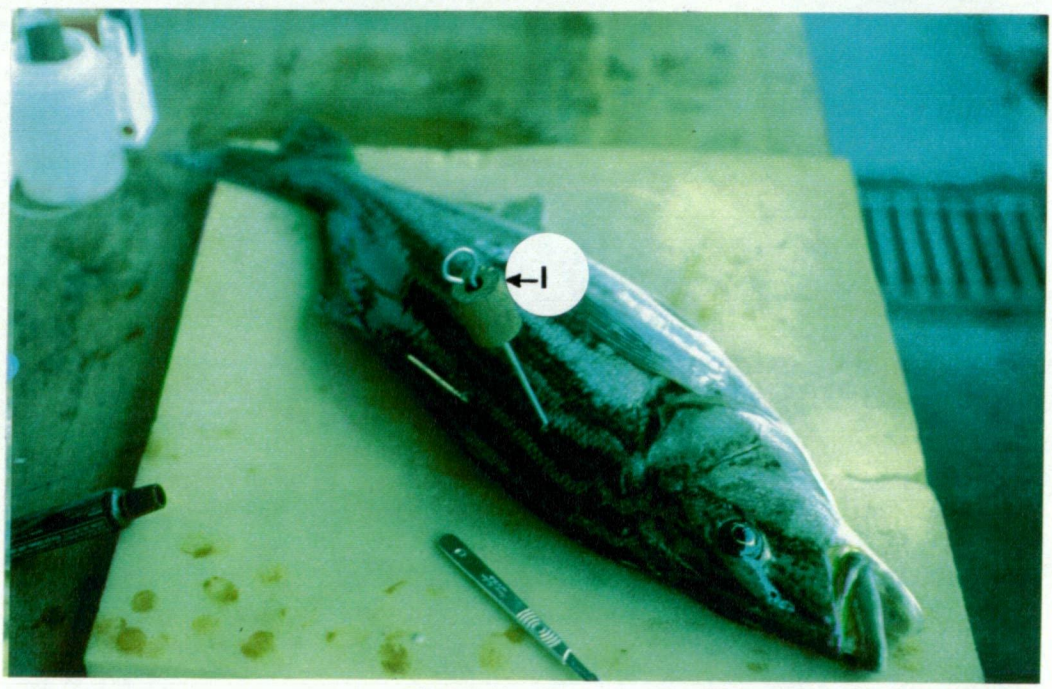


Figure 6.8 Implantation of sustained release hormone implants into anterior dorso-lateral musculature of *Latris lineata* using implantation device (I).

Figure 6.9 Implanted *Latris lineata* which died two months after trials, showing healing of the point of implantation (I).

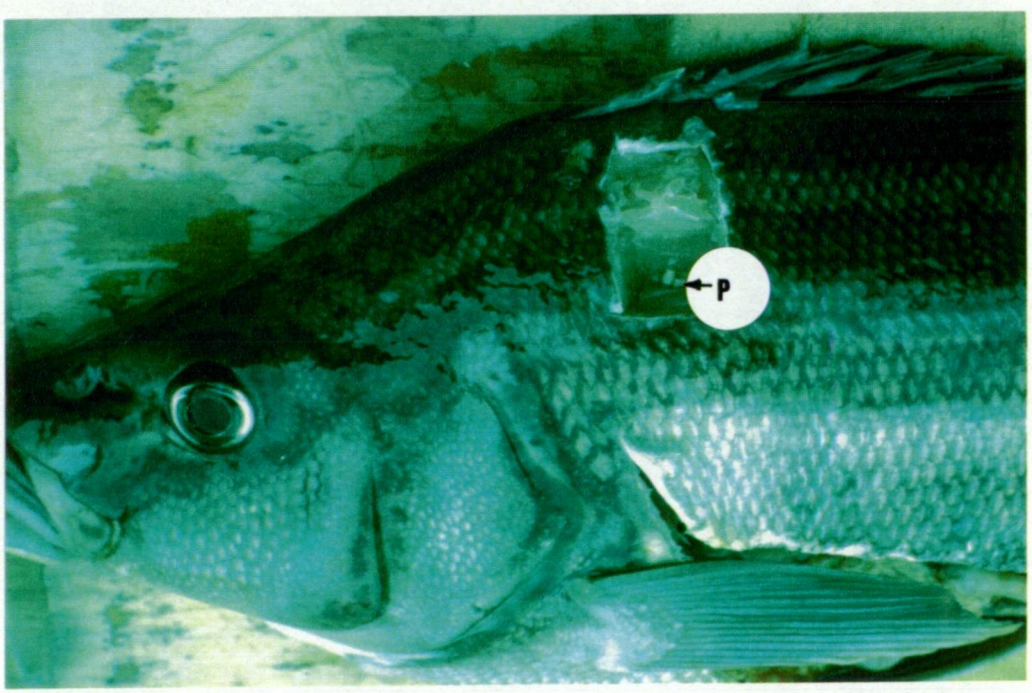


Figure 6.10 Position at which pellets are embedded (P) within the anterior dorso-lateral musculature of *Latris lineata*.

implantation incision, blood sampling point and any minor abrasions. The fish was returned to the holding tank or cage were it was observed until recovery. The complete procedure following anaesthesia took approximately 3-4 minutes for each fish. When blood samples had completed clotting (1-2 hours) they were centrifuged at 3000 G for 10 minutes. Serum was withdrawn in 300µL aliquots using a micropipette . Each aliquot was transfered into a seperate 1.5 ml Eppendorf tube which was then capped and labelled. Three 300µL aliquots of serum were taken from each blood sample. All tubes were stored frozen at -86 °C until sex steroid assays were conducted.

6.3.3 Sex steroid assays.

Levels of 17β-estradiol and testosterone (17β-hydroxy-4-androsten-3-one) were determined using Orion Diagnostica Spectria coated tube radioimmunoassay (RIA) kits developed for use with human serum. Assay procedures followed the directions provided. Following incubation tubes were counted using an LKB Wallace 1261, 20 detector (^{125}I) gamma counter. Counts of known standards were spline fitted using the RIACalc data handling package. The standard curve generated was used to determine steroid concentration (NM/L*) from raw sample counts. Duplicate assays were conducted for each serum sample, for each steroid, and the mean of the two values was used in analyses. One aliqout of serum (300 µL) from each sample was freeze dried at Mt. Pleasant Laboratories (Tasmanian Department of Primary Industries; Launceston). RIA of these sample replicates was undertaken at the Queen Elizabeth Hospital (QEH), Adelaide (Reproductive endocrinology section) to provide a back-up for results obtained at UTL.

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* Conversion of NM/L to ng/ml:
17β-Estradiol (ng/ml) = 17β-estradiol (NM/L) x 0.2724
Testosterone (ng/ml) = Testosterone (NM/L) x 0.288

6.3.4 1992 DSF sustained release hormone implant trial.

The DSF experiment commenced on 14 July. Fish selected for the trial came from broodstock which had been maintained in a 7 m^3 outdoor tank receiving flow through seawater at ambient temperature. The experimental design (as follows) required 24 females. However, gonad biopsy samples taken using an endometrial biopsy device (Laboratoire CCD, Paris), showed that only 23 of the available females had oocytes past the primary stages of development (mature females). As a consequence one control replicate was dropped from the experiment at this stage, leaving the following number of treatments and replicates;

Treatment	Females	Males.	
High dose	8	4	
Low dose	8	4	
Control	7	4	
Totals	**23**	**12**	**= 35.**

After receiving the first implant all fish were transported to the DSF net cage (5m x 5m x 5m) at the Aquatass Pty. Ltd. marine farming lease because of a need for tank space for incoming broodstock at DSF Taroona laboratories. Fish received the second implant (11 August) at this location and were moved back to the outdoor tanks one week before the third implant (8 September) to allow fish to be observed regularly after this time. Only one fish showed external signs of ovarian maturation and the trial was discontinued at the end of September for logistical reasons.

Mean oocyte diameters were determined from ovarian biopsy samples preserved in 4% FBSW. Histological slides were prepared from samples with predominantly primary growth oocytes. Embedded samples were sectioned to 4 μm and conventional heamatoxylin and eosin staining followed. Oocytes were measured

using a stage microscope with an ocular micrometer. Samples with latter oocyte stages were measured directly using a binocular microscope with an ocular micrometer. In both procedures the 20 largest oocyte were measured and the 10 largest oocytes were used to determine mean oocyte diameter for the sample.

Mean oocyte diameters for samples taken from all fish at each implanting date are provided in Appendix 22a together with means (+/- standard error) for each treatment which are shown graphically (+/- standard deviation) in Appendix 22b. The F-max test (Sokal and Rohlf, 1973) confirmed homogeneity of variance for the mean oocyte diameter data. Two way ANOVA of treatment and implant number (month) shows no significant difference ($P > 0.05$) in mean oocyte diameters between treatments (Appendix 23a) however a significant effect ($P < 0.05$) is shown for the month of implantation. If implant treatment is disregarded one way ANOVA and comparison of means (Fisher PLSD), for all fish, shows that oocyte diameter is significantly different ($P < 0.05$) between all months (Appendix 23b). This result demonstrates that significant oocyte development has occurred between each implanting date.

These results are presented graphically in Figure 6.11 which shows that the *Latris lineata* receiving the high dose treatment appear to have had the largest mean oocyte diameter by the time of administering the third implant. However, ANOVA has shown that this diameter was not significantly ($P > 0.05$) greater than the mean oocyte diameter of fish receiving the low dose and control implant treatments. All treatments followed a similar pattern until the second implant. In both hormone treatments the dosage of LHRHa was increased markedly at the second implant. At this time the dosage of LHRHa was increased from 25 µg/kg b.w., to 100 µg/kg b.w., in the low dose treatment,while the dosage in the high dose treatment increased from 50 µg/kg b.w. initially, to 200 µg/kg b.w., in the August and September implants. Separation of the two hormone treatments following the

August implant may indicate that although the difference is not significant at this stage, the high dosage appears more likely to have further promoted oocyte development in *Latris lineata*, had the trial had been continued.

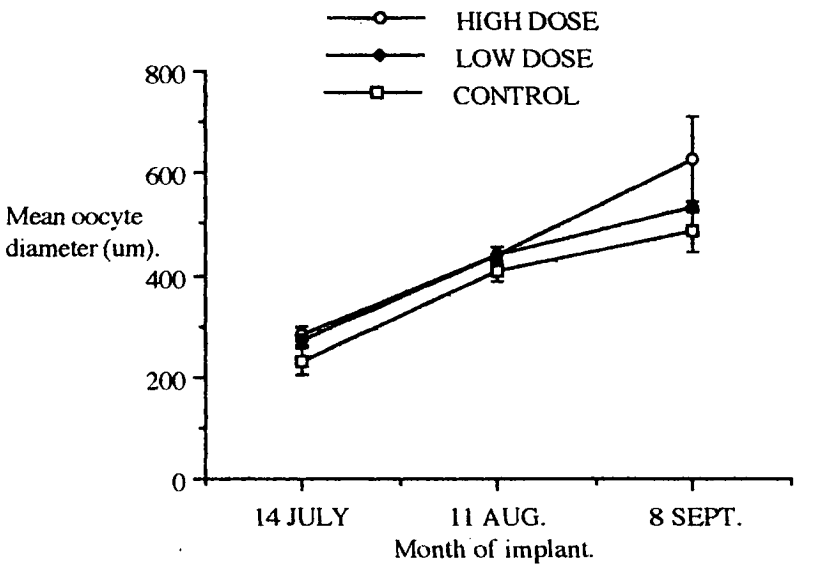


Figure 6.11 Mean oocyte diameter (+/- standard error) of *Latris lineata*, at each implanting date during the 1992 DSF sustained release hormone implant experiment.

Within the high dose treatment one female had mature, hydrated eggs at the time of the September implant. Although showing considerable abdominal swelling no eggs could be manually stripped without causing the ovary to distend from the vent. Examination of the September (final) results from individual fish within each treatment (Appendix 22a) shows that 87.5 % (7/8) of high dose implanted fish, and 85.7% (6/7) of low dose implanted fish, had mean oocyte diameters exceeding 500 µm. Only 20% (1/5) of control fish had mean oocyte diameters greater than 500 µm. These results indicate that at the time of the third implant being administered, hormone treated fish had mean oocyte diameters consistently greater than 500µm. Histological observations (section 4.5) indicate that vitellogenesis is completed when the mean oocyte diameter of oocytes reaches 605.3 +/- 36.6 µm. Results from the 1991 hormone induction investigation suggest that the mean

oocyte diameter needs to exceed 600 μm to maximise the success of any acute spawning induction methods which could be employed, when oocytes had attained this critical diameter. The high dose pellet implant protocol appears to be the most likely formulation capable of achieving this objective in *Latris lineata*.

Results from RIA for 17β-estradiol and testosterone levels in serum samples are presented in Appendices 24a and 24b respectively. Treatment means and variance data are shown in Appendix 25. One batch of serum from the second implant could not be accounted for at the time of RIA analysis. Many of the UTL testosterone assays for the August and September implants returned counts outside the range of the assay standards and could not be repeated as insufficient serum was left over to repeat the assay. Consequently UTL 17β-estradiol results were used for all analyses, while testosterone data used is a combination of results from UTL (July implant) and QEH (August and September implants) assays.

Within treatments large inter-replicate variance (Appendix 25) was common. Appendices 26a and 26b show graphically the degree of variablity of raw data for serum 17β-estradiol and testosterone respectively, for each treatment. The F_{max}-test (Sokal and Rohlf, 1973) confirmed the existance of heterogeneity of variance within the data. Several transformations were applied to the steroid data however the homogeneity assumption for ANOVA could not be met. Consequently the non-parametric Kruskal-Wallis test was used to analyse the effect of experimental treatments on steriod levels at the time of each implant.

Mean serum levels of 17β-estradiol and testosterone, for each treatment, are shown in Figures 6.12 and 6.13 respectively. No significant effect ($P > 0.05$) due to any implant treatment was shown for either steroid, at any monthly implant (Appendix 27). Although it appears graphically that differences may exist between implant treatments and between different months, the variance of the data was

shown to increase dramatically with rising mean serum steroid levels (Appendix 25). This situation may have reduced the ability of the non-parametric analyses to distinguish any treatment effects.

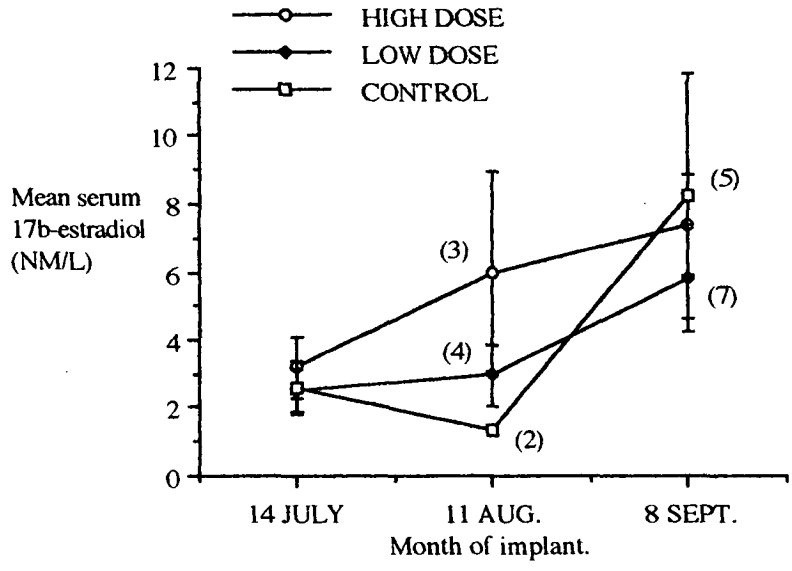


Figure 6.12 Mean levels of serum 17β-estradiol (+/- standard error) for *Latris lineata*, at the time of each monthly implant, during the 1992 DSF sustained release hormone implant experiment.

Control n=7; High/Low n=8, unless otherwise indicated (n)

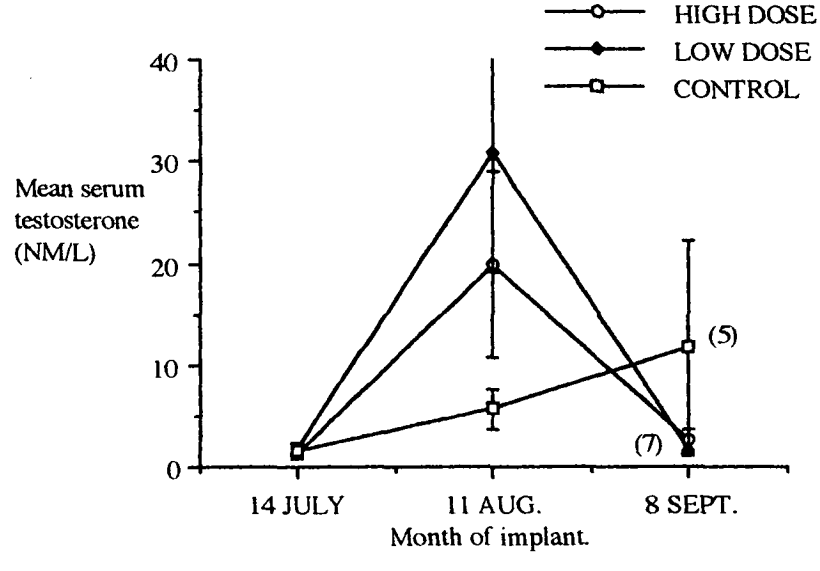


Figure 6.13 Mean levels of serum testosterone (+/- standard error) for *Latris lineata*, at the time of each monthly implant, during the 1992 DSF sustained release hormone implant experiment.

Control n=6; High/Low n=8, unless otherwise indicated (n)

Results for the 24 hr changes in17β-estradiol and testosterone following each implant are presented in Figure 6.14. These figures demonstrate that the mean serum sex steroid levels recorded 24 hours after implantation generally follow those recorded at the time of implantation (0 hr). The heterogeneity of the data necessitated the use of the non-parametric Mann-Whitney U test to investigate the implant induced changes in steroid levels at 24 hours following each monthly implant (Appendix 28). Results of this analysis reveal that the mean serum testosterone level of fish implanted with the high dose pellets in July, fell significantly ($P > 0.05$) from 1.19 NM/L initially, to 0.18 NM/L at 24 hours after implanting. Mean serum testosterone of control fish showed a significant increase ($P < 0.05$), from an initial level of 1.65 NM/L, to 3.97 NM/L over the same period. Other than these results, no other significant changes in mean serum levels of 17β-estradiol and testosterone in female *Latris lineata*, in the 24 hour interval following implanting, have been demonstrated for the experimental treatments used in this investigation (Figure 6.14).

Due to the large amount of variability in these data, no clear conclusions can be drawn from the results of the DSF experiment. Although the high dose hormone treatment provided fish which appeared to have had the highest mean oocyte diameter at the time of the final implant, this difference was not significantly greater than the mean oocyte diameter recorded for low dose and control fish. Mean serum 17b-estradiol levels follow a similar pattern over the duration of this investigation, for both implant treatments, neither of which are significantly different from control fish, at any implant date. No significant difference has been established between the mean serum testosterone levels of the experimental treatments employed. The pattern shown for mean serum testosterone of both groups of hormone implanted fish displays a similar profile, which appears to differ from that of control fish. However, this has not been shown by statistical analysis.

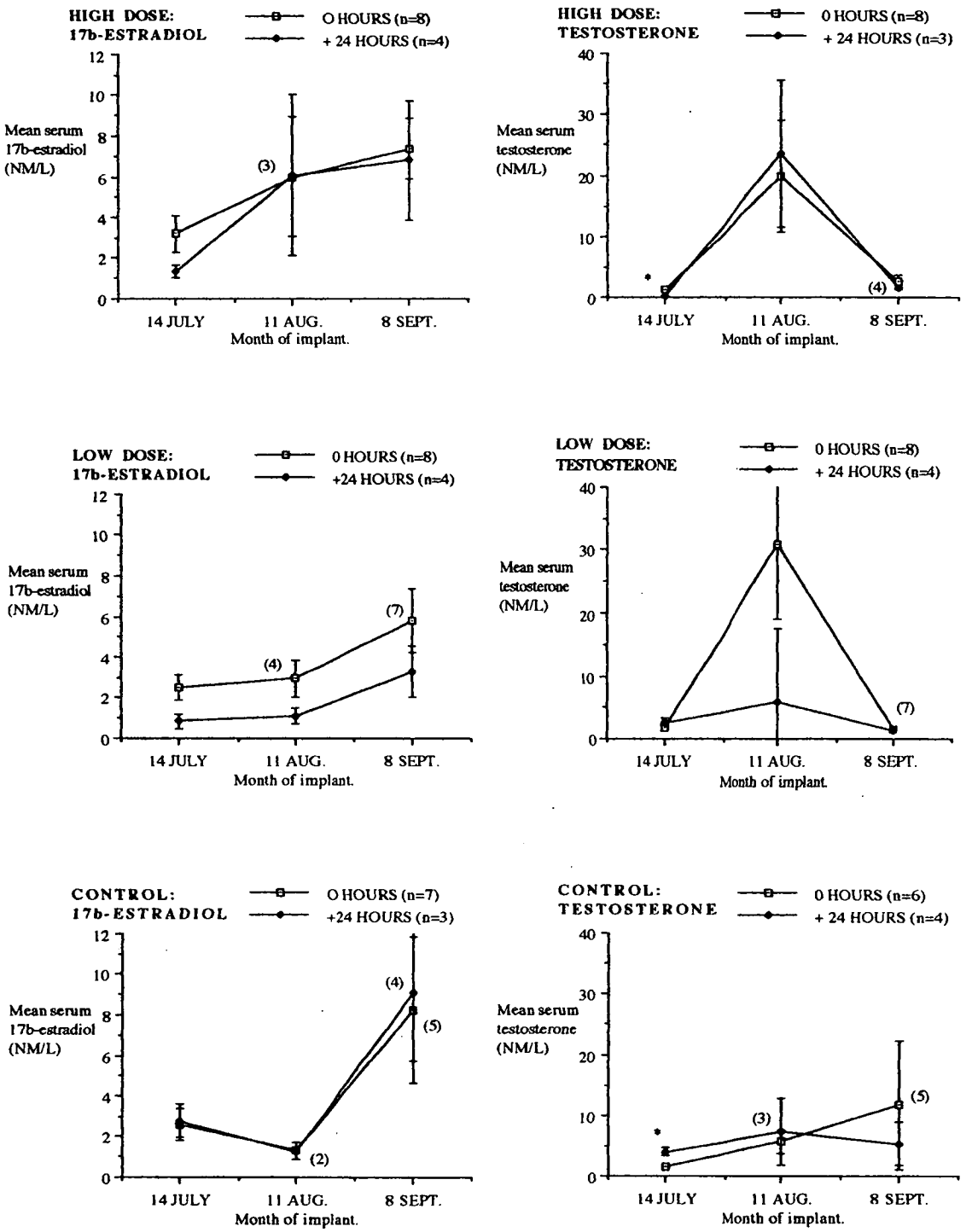


Figure 6.14 24 hour changes in mean serum levels of 17β-estradiol and testosterone (+/- standard error) for *Latris lineata*, for each experimental treatment, after each monthly implant. (* = significantly different at $P < 0.05$; N as stated unless otherwise indicated (n) on graphs)

Accepting the statistical limitations of these results a descriptive analysis of the data presented in Figure 6.14 suggests that in both hormone implant treatments there was aa apparent increase in mean serum testosterone levels between the July and August implants (Figure 6.13). An apparent decline in mean serum testosterone levels was subsequently observed between the August and September implants. The control treatment did not show this pattern of pronounce rise and decline in levels of mean serum testosterone. It is suggested that this steroid profile displayed by placebo implanted fish is characteristic of female teleosts approaching spawning (section 5.3.1.). Although not statistically significant, these apparent variations between the mean serum testosterone profiles of hormone implanted and control fish, can be interpreted based on the current understanding of reproductive endocrinology in teleosts (chapter 5).

In the two-cell model for steroid production by the follicle, testosterone produced by the thecal cells is transfered to the granulosa cells and aromatized to 17β-estradiol (Kagawa *et al.*, 1982). In the current study, it is suggested that the elevated mean serum testosterone levels of hormone implanted females, recorded at the time of the August implant, could reflect the limited ability of the follicles of developing oocytes of *Latris lineata*, to aromatize testostosterone. Young *et al.* (1983a) contend that changes in plasma testosterone levels reflect the the use of this steroid as a substrate for the production of 17β-estradiol. Figures 6.15a and 6.15b demonstrate the relationship between mean levels of serum sex steroids and mean oocyte diameter recorded during this study, for hormone implanted and control *Latris lineata* respectively. In these figures implanting month are ignored.

In the current study, the source of serum testosterone recorded has not been proven. Results from individual hormone implanted fish demonstrate that elevated levels of serum testosterone were recorded in 7 hormone implanted females at the time of the August implant (Figure 6.15a). Mean oocyte diameter data (Appendix

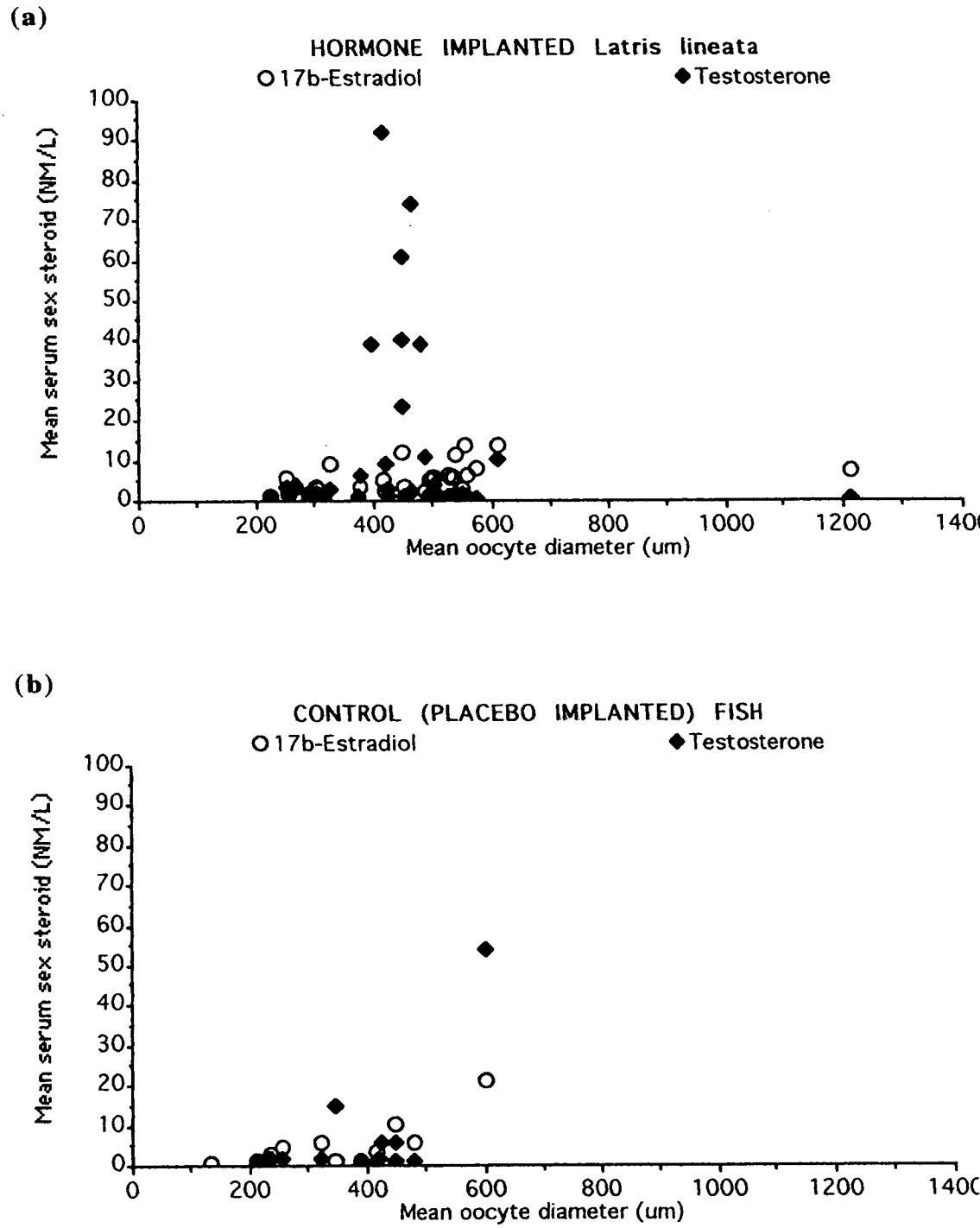


Figure 6.15 Relationship between mean serum levels of 17b-estradiol and testosterone, and mean oocyte diameter of hormone implanted and control *Latris lineata*, during the 1992 DSF sustained release hormone implant trial.

22a) confirms that oocytes of these fish were at the early vitellogenic stage of development at this time (mean oocyte diameters ranging from 399.0 - 463.8 μm). Figure 6.15b shows that one control fish recorded an elevated level of testosterone at the late vitellogenic stage of oocyte development (mean oocyte diameter 600.2 μm). This observation indicates that the hormone implants have induced elevated levels of serum testosterone in 7 of the individual *Latis lineata* used in this investigation.

If it is accepted that 17α-MT released from pellet implants stimulates pituitary GtH production (Crim and Evans, 1979, 1983) and enhances pituitary responsiveness to GnRH (Trudeau, 1993), then the elevated serum testosterone levels recorded by RIA is most likely to have resulted from GtH stimulated testosterone production. (cross reactivity of the testosterone antiserum used in the RIA procedure to methlytestosterone = 0.45%). It is suggested that the elevated mean serum testosterone levels in some hormone implanted fish (n=7) reflects the limited ability of these fish to aromatise testosterone to 17β-estradiol, during the early stages of vitellogenesis. If the two-cell model for steroid production by the follicle of *Latris lineata* is accepted then Figure 6.15a suggests that the capacity of oocyte follicles of *Latris lineata*, to aromatize testosterone to 17b-estradiol may increase during vitellogenesis, particularly following the attainment of a diameter of approximately 500 μm. This observation may explain the elevated levels of testosterone recorded for some (n=7) of the hormone implanted fish, at the time of the August implant.

This interpretation is in agreement with *in vitro* studies on follicles from amago salmon, *Oncorhychus rhodurus*, in which the ability of granulosa layers to produce 17β-estradiol was shown to increase during vitellogenesis (Kagawa *et al.*, 1982; Young *et al.*, 1983a). This increase was attributed to a GtH induced increase in the capacity of follicular thecal cells to produce testosterone, and and an increase

in aromatase activity of granulosa cells (Kanamori *et al*, 1988a). A similar situation occurs in the daily spawning medaka, *Oryzias latipes*. In this species the ability of follicles to produce 17β-estradiol increases as follicles develop from early, to late vitellogenesis. A rise in aromatase activity is closely associatated with the ability of these follicles to produce 17β-estradiol (Sakai *et al.*, 1988).

A compounding factor in this explanation is the change in hormone dosages which accompanied the decline in mean serum testosterone values of hormone implanted fish following the August implant. The hormone dosages used in the July implants were 50 μg LHRHa + 200 μg 17α-MT/kg b.w., for the high dose treatment, and 25 μg LHRHa + 100 μg 17α-MT/kg b.w., for the low dose treatment. The dosages of LHRHa used in hormone implants was increased for the August and September implants. The high dose treatment was increased to 200 μg LHRHa + 200 μg 17α-MT/kg b.w., while the low dosage treatment was increased to 100 μg LHRHa + 100 μg 17α-MT/kg b.w. This change in LHRHa dosage for hormone implanted fish corresponds with the decline in mean serum testosterone levels recorded following the August implant. As such, the effect of an increased dosage of LHRHa cannot be discounted in any interpretation of these results.

The current understanding of the effects of LHRHa on circulating levels of sex steroid oocyte development (chapter 5) contends that this increase in LHRHa dosage may ulimately result in an increase in GtH production by pituitary. In this investigation it appears that the increase in dosage of chronically administered LHRHa, coincided with a decrease in mean serum testosterone levels. Such a response is in agreement with the two-cell model for GtH induced steroid production by the oocyte follicle. A tentative interpretation would be that the increased dosage of LHRHa (in combination with 17α-MT) used in the August implants was sufficient to stimulate GtH induced activation of aromatase, thus increasing the utilization of testosterone as a substrate for 17β-estradiol production

by mid-late vitellogenic oocytes, with a concommitent decline in the mean serum testosterone levels observed.

Results of previous research relevant to this explanation of the current results are conflicting. Kagawa *et al.* (1982), Young *et al.* (1983a) and Kanamori *et al.* (1988a) found no significant increase in the production of 17β-estradiol by granulosa cells of amago salmon (incubated *in vitro* with testosterone), in response to endogenous GtH, at any stage of follicular development. Kagawa *et al.* (1982) contend that all that is needed for granulosa cells to synthesize 17β-estradiol is the availability of testosterone.

More recently Sakai *et al.* (1987, 1988) found that capacity of medaka follicles to produce 17β-estradiol in response to partially purified chum salmon gonadotropin or pregnant mare's serum gonadotropin, was strongly correlated with vitellogenic development of oocytes. This supports the interpretation of the results from the current study, which are founded on the notion that LHRHa stimulated GtH secretion stimulates follicular production of 17β-estradiol. It is suggested that the under the influence of appropriate dosages of LHRHa, administered as a sustained release hormone implant, GtH secretion is stimulated, and the capacity of follicles of *Latris lineata* to produce 17β-estradiol consequently increases during the latter stages of vitellogenesis. Confirmation of this scenario would require determination of circulating levels of GtH prior to, and following the change in dosage of the hormone implants used.

6.3.5 1992 UTL sustained release hormone implant trial.

The objective of this experiment was to induce spawning of captive *Latris lineata* and to follow the change in serum sex steriods, at 48 hourly intervals, following implantation of broostock with sustained release hormone pellets. The replication

possible in this experiment was limited by the volume of the holding tank ($4m^3$) which allowed only 12 female and 4 male fish to be accommodated. Fish used in this investigation were supplied by DSF. Selected fish were transported from Hobart to Launceston, one month prior to the first implanting date. Fish were fed once daily to satiation with chopped jack mackeral, *Trachurus declivis*, and squid. Feed was withheld for one day prior to each implanting date.

As water temperature and photoperiod are the major physical environmental factors controlling the reproduction of temperate fish (Billard *et al.*, 1981, Lam, 1983), these factors were controlled in this indoor investigation. Water temperature was maintained at 13 °C and a photoperiod of 10 hr light (L):14 hr darkness (D) was employed until the start of this investigation. Water temperature was increased to 13.5 °C when the first implant was administered, and the light duration was increased by 30 minutes approximately every 14 days after this date, until a 12 hr L: 12 hr D photoperiod was reached at the time of the final implant.

Pellets were prepared and implanted as previously described (sections 6.3.1 and 6.3.2 respectively). Implants were administered on 17 July (implant 1), 13 August (implant 2), and 12 September (implant 3). At the time of each monthly implant three control females were implanted with placebo pellets containing no hormones. On these dates 9 female and the 4 male fish were implanted as follows, using the high dose implant protocol employed in the complementary DSF investigation:

Implant no./date	Hormone implant treatment	Control-placebo implanted
1. 17 July '92	50 μg LHRHa	10 mg/kg b.w. pellets
	+ 200 μg 17α-MT/kg b.w.	without hormones
2. 13 Aug.'92	200 μg LHRHa	10 mg/kg b.w. pellets
	+ 200 μg 17α-MT/kg b.w.	without hormones
3. 12 Sep.'92	200 μg LHRHa	10 mg/kg b.w. pellets
	+ 200 μg 17α-MT/kg b.w.	without hormones

On each implanting date an oocyte sample was taken from all females by ovarian biopsy. A blood sample was also taken from all fish at this time (0 hr). Further blood samples were taken from 3 hormone implanted females at 48 hr intervals. Three different females were sampled at each 48 hr interval until 144 hr (6 days) post implantation, at which time blood was sampled from the 3 remaining hormone implanted females, and the 3 control females. To allow identification all fish were anchor tagged 1-2 cm below the second dorsal spine. A 5 - 7 mm section of coloured plastic tubing filled with silicone sealant was pre-fixed to the end of each tag used. Three females were randomly assigned a similar coloured tag so that blood samples could be taken from this sub-group at the designated 48 hr interval, as shown in table 6.2. This tagging method enabled visual identification of fish to be sampled prior to capture, (i.e. by tag colour) and allowed anaesthetized fish to be distinguished individually by tag number.

The blood sampling regime followed is shown in Table 6.2 which reveals that 2.0 - 2.5 ml of blood was taken from each female, on two occasions per month. This limited sampling procedure was adopted to minimise the adverse effects of handling stress during frequent sampling, which may have compromised the effects of the hormone implants. Unfortunately, following this procedure only a small number of blood samples (n=3) were collected at each 48 hr interval.

Two of the females implanted with the hormone treatment were found to be immature, as only primary stage oocytes were present throughout the investigation. No initial biopsy sample could be obtained from one control fish and it was inferred that it was an immature male. These three immature fish would not have been used if replacement fish were available at the time of commencing the investigation. Consequently a *post priori* decision was made to delete them from all analyses of the data.

Table 6.2 Blood sampling protocol followed in 1992 UTL investigation of the use of sustained release hormone implants for inducing spawning of female *Latris lineata*.

		Time following implantation (Hours)			
Treatment	Fish No.	0*	48	96	144
Implanted	300	+	+	-	-
	301#	+	+	-	-
	302	+	+	-	-
	303	+	-	+	-
	304	+	-	+	-
	305	+	-	+	-
	306#	+	-	-	+
	307	+	-	-	+
	308	+	-	-	+
Control	313#	+	-	-	+
	314	+	-	-	+
	315	+	-	-	+

+ = Blood sample taken.
* = Oocyte sample taken from all fish.
# = Fish deleted from analysis.

Mean oocyte diameter data is provided in Appendix 29 and presented graphically in Figure 6.16. Mean oocyte diameter data was homogeneous (F-max test) and two way ANOVA (Appendix 30a) showed that the month of implanting significantly effected ($P < 0.05$) mean oocyte diameter, as would be expected. Mean oocyte diameter was not significantly ($P > 0.05$) affected by the hormone implant treatment. One way ANOVA and comparison of means (Fisher PLSD), for hormone implanted fish, showed that mean oocyte diameter was significantly greater ($P < 0.05$) in August and September, than in July (Appendix 30b).

Similar one way ANOVA for control fish shows a significantly greater ($P < 0.05$) mean oocyte diameter in September than in July. In this analysis no significant difference ($P > 0.05$) in mean oocyte diameter was found between either July and August, or August and September implants (Appendix 30c).

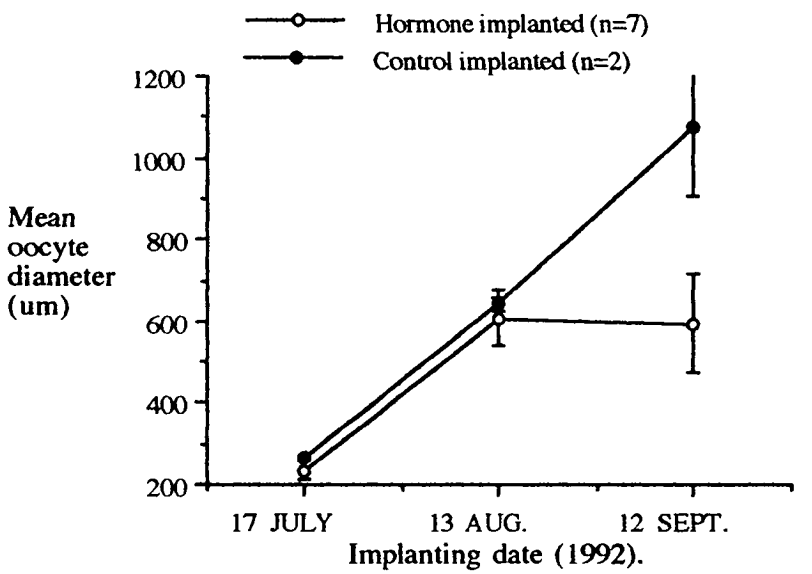


Figure 6.16 Mean oocyte diameter (μm) +/- standard error, for hormone implanted and control female *Latris lineata* at the time of each monthly impalnt during the 1992 UTL sustained release hormone trial.

Prior to the 1992 investigation a filter mat (Dacron^R filler material on a plastic mesh support) was fitted to the outlet of the holding tank at UTL. This filter improved water quality of the holding system and also served to concentrate eggs spawned within the tank. On 10 August 1992, three days before the second implanting date, a quantity of eggs were found in the outlet filter. Eggs were not fertilized and were discarded. At the time of administering the second implant a small quantity of mature eggs were stripped from one of the hormone implanted fish (fish no. 305) and it is inferred that this fish may have been responsible for the spawning recorded. This is the first record of spontaneous release of eggs by captive *Latris lineata*. Spontaneous egg release was recorded on two other occasions during this investigation, suggesting that with better

holding facilities and a greater number of male fish, this may be achieved more consistently.

Once spontaneous egg release was recorded females were observed more closely and those displaying external signs of oocyte maturation were removed from the tank and anaesthetized, to check for the presence of free flowing ovulated eggs. Records of eggs collected from females (Table 6.3) shows that two implanted fish (fish numbers 304 and 305) each ovulated multiple batches of oocytes. Each of the two control females spontaneously released a single batch of eggs on seperate occasions. This was inferred from the large mean oocyte diameters recorded for these fish, together with the observation that the visually obvious abdominal swelling present on the preceeding day had contracted at the time that eggs were collected in the outlet filter.

The volume of each batch of manually stripped eggs was determined before fertilization, using a measuring cylinder. Time did not allow counting of each individual batch of eggs as larval rearing was also being pursued at this time. However, a representative sample of unfertilized eggs was counted using a stereo-microscope. Five 1 ml aliquots of eggs were dispensed with a wide bore 1 ml graduated glass pipette, providing a mean of 619 +/- 19.7 eggs/ml. This figure was used to quantify the approximate number of eggs collected (rounded to nearest 1000) included in Table 6.3.

Figure 6.17 shows the frequency of spawnings recorded during this investigation. One implanted fish (305) ovulated a batch of eggs 21 days before the first control fish (314) spontaneously released a batch of eggs. Another implanted fish (304) ovulated a batch of eggs five days latter, so that 2 of the 7 mature implanted fish were producing successive batches of eggs during a 16 day period before the first ovulation by a control fish. The batch of approximately 110,000 eggs stripped from fish number 305 on 24 August 1992 was used for investigations into larval rearing. Following on from

Table 6.3 Records of eggs manually stripped, or spontaneously released in the holding tank, during the 1992 UTL sustained release hormone implant investigation.
(H = hormone implanted fish; C = control fish; *= implanting date)

	Fish number: Volume (ml) eggs collected (approx. total no. eggs) or description.			
DATE	304 (H)	305 (H)	314 (C)	315 (C)
10 Aug.	-	Spontaneous egg release in tank?	-	-
13 Aug.*	-	10 ml (6,000)	-	-
15 Aug.	126 ml (78,000)	103 ml (64,000)	-	-
17 Aug.	Small amount of over-ripe eggs	Ovarian fluid and over-ripe eggs	-	-
19 Aug.	140 ml over-ripe	-	-	-
20 Aug.	-	40 ml over-ripe	-	-
22 Aug.	80 ml over-ripe	120 ml over-ripe	-	-
24 Aug.	-	110 ml (68,000)	-	-
25 Aug.	60 ml over-ripe	-	-	-
26 Aug.	70 ml (43,000)	-	-	-
27 Aug.	-	140 ml (87,000)	-	-
28 Aug.	-	20 ml over-ripe	-	-
29 Aug.	90 ml (56,000)	-	-	-
30 Aug.	-	70 ml (43,000)	-	-
1 Sept.	70,000 (43,000)	25 ml over-ripe	Spontaneous egg release in tank ?	-
2 Sept.	-	40 ml (25,000)	30 ml over-ripe	-
4 Sept.	50 ml (31,000)	20 ml over-ripe	-	-
5 Sept.	-	5 ml over-ripe	-	Spontaneous egg release in tank ?
7 Sept.	20 ml poor quality	-	-	-
12 Sept.*	10 ml over-ripe	-	-	Over-ripe eggs
14 Sept.	10 ml (6,000)	-	-	-

experiences gained over 2 previous larval rearing seasons (1990, 1991), these eggs ultimately developed to produce the first recorded successful rearing of juvenile *Latris lineata*. This information will be reported in future papers.

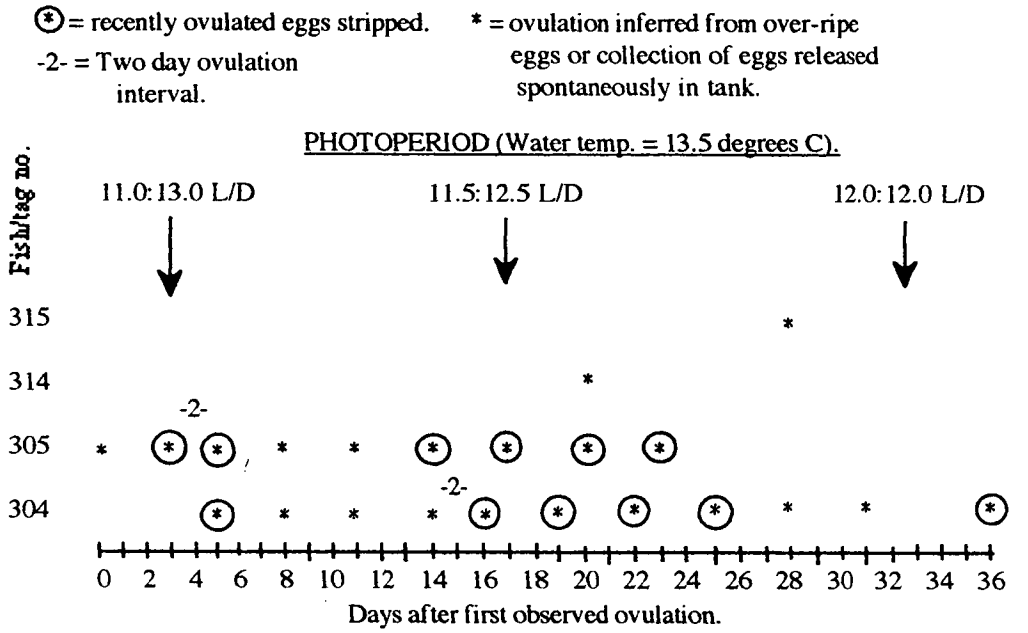


Figure 6.17 Recorded and inferred ovulations of implanted (304, 305) and control (314, 315) *Latris lineata* during the 1992 UTL sustained release hormone implant trial.

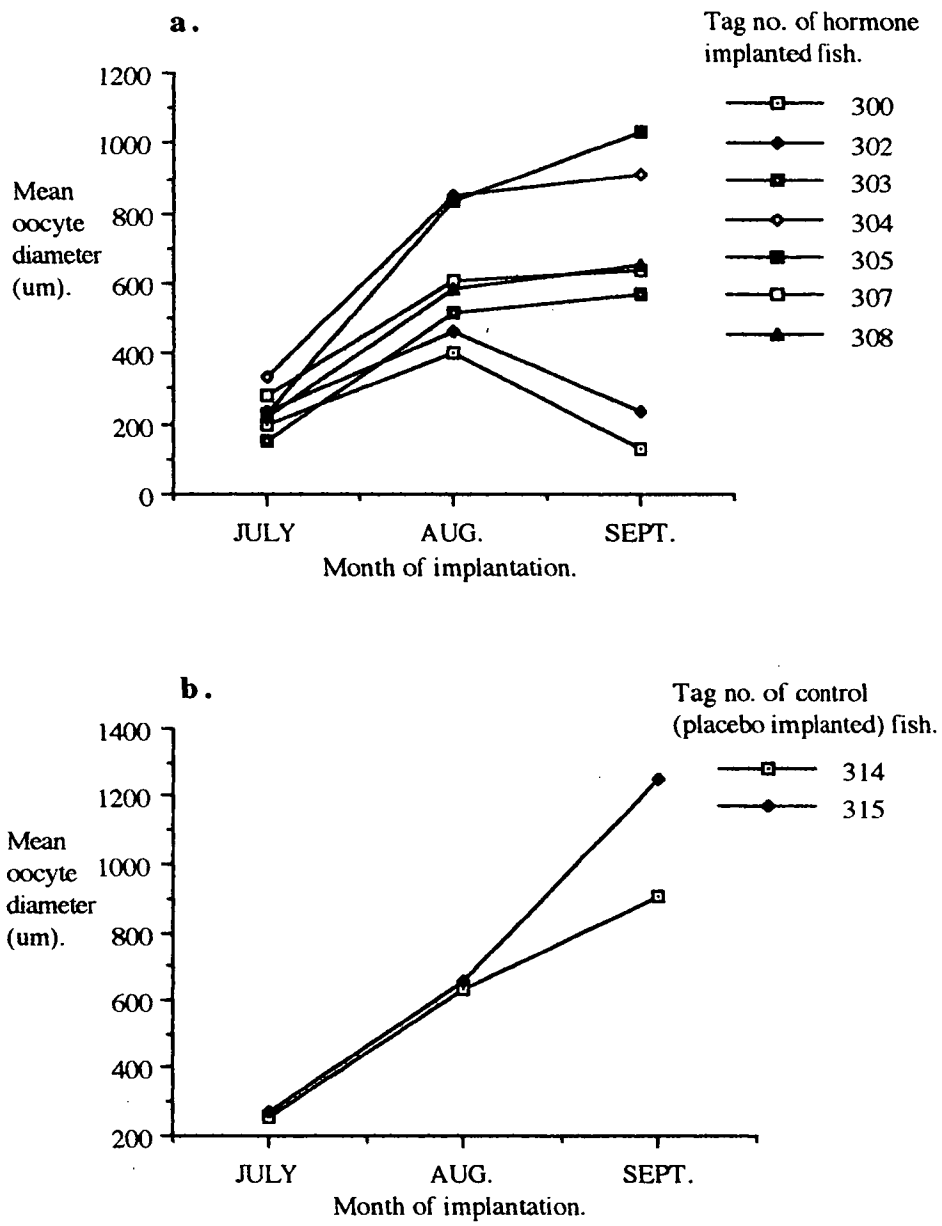
In Figure 6.17 it can be seen that one implanted fish (304) ovulated at least 11 batches of eggs during this investigation. Another implanted fish (305) ovulated at least 9 batches of eggs. In both fish successive ovulations were mostly seperated by a 3 day interval although on at least one ocassion for each fish a 2 day interval seperated ovulations. Both control fish ovulated a single batch of eggs and showed no signs of maturation of further batches of oocytes. These observations support the conclusion reached in section 4.5, that female *Latris lineata* are capable of ovulating multiple batches of oocytes, which develop in a group synchronous manner during the spawning season.

Results presented suggest that the sustained release hormone implant treatment advanced the spawning period, and assisted the maintenance of the pattern of multiple spawning by 2 of the 7 hormone implanted fish. Oocytes samples from the remaining 5 hormone implanted fish (Figure 6.18a) show that following the second implant (August) two fish responded adversely to implantation. As in the DSF experiment the dosage of LHRHa was increased at this time to from 50 mg/kg b.w., to 200 mg LHRHa/kg b.w. The remaining 3 hormone implanted fish continued to complete vitellogenesis. However oocytes did not progress into final maturation stages of development.

As described in section 5.4.4.2 the use of sustained release hormone implants has proven successful in advancing the spawning period of a number of species of teleosts, including rainbow trout (Crim *et al.*, 1983b), Atlantic salmon (Weil and Crim, 1983; Crim and Glebe, 1984), herring (Carolsfeld *et al.*, 1988), striped mullet (Tamaru *et al.*, 1989) and barramundi/seabass (Harvey *et al.*, 1985; Kuo, 1991). Multiple spawning of hormone implanted fish has also been reported for milkfish (Marte *et al.*, 1988), barramundi/seabass (Almendras *et al.*, 1988; Garcia, 1989; Kuo, 1991) and striped mullet (Tamaru *et al.*, 1989).

The pattern of spawning observed for captive *Latris lineata* is comparable to that described for captive cod, *Gadus morhua*, and milkfish, *Chanos chanos*. Kjesbu (1989) demonstrated that among 18 captive female cod which spawned naturally at least once (5-8 °C water temperature), one female released 19 batches of eggs over a 47 day interval, with a mean spawning interval of 61.8 hours. Tamaru *et al.* (1988) reports up to 11 spawning attempts for milkfish induced to spawn with varying dosages of i.m. injected LHRHa. Although only 28.6% of implanted *Latris lineata* ovulated batches of eggs, these results indicates that further investigations of the use of sustained release hormone implants could advance the spawning period of a higher proportion of hormone implanted broodstock, and maintain multiple spawning of

these females. Such an outcome would assist further research on larval rearing of *Latris lineata* and would assist the viable operation of a commercial marine fish hatchery.



Figures 6.18 Mean oocyte diameter (μm) of each implanted *Latris lineata* at each implanting month during the 1992 UTL sustained release hormone implant trial. **a**. Hormone implanted fish. **b**. Control fish.

The F-max test for heteroscedacity (Sokal and Rohlf, 1973) was applied to all serum sex steroid data (Appendix 31). Sporadic heterogeneity of this variance was confirmed to exist within the replicates from the 48 hr time intervals, and within treatments. Transformation of data did not fulfil the assumption of homogeneity of variance required for ANOVA. The small number of replicates remaining after deletion of two implanted females and one control female (0 Hr v 48Hr, n=2; 0 Hr v 96Hr, n=3; 0 Hr v 144Hr, n=2) did not allow the use of non-parametric methods for analysis of the changes in serum sex steroid levels. Consequently ANOVA of $\log_{10}$ tranformed serum sex steroid data was reverted to as it is regarded as a robust method (Romano, 1977) and when its assumptions hold approximately it is generally more efficient for determining departures from the null hypothesis (Sokal and Rohlf, 1981).

Serum levels of 17β-estradiol and testosterone recorded at implanting and at 48 hr intervals following each monthly implant are presented in Appendix 31. Mean serum levels of 17β-estradiol and testosterone, for hormone implanted and control fish, are presented in Figures 6.19a and 6.19b respectively. A two-factor repeated measures ANOVA would normally be used to analyse data resulting from the repeated sampling of specific fish undertaken in this investigation. The small number number of replicates remaining after deletion of two implanted females and one control female negated the use of this method for analysis of these data.

Using implanting month as an experimental treatment, two way ANOVA using $\log_{10}$ transformed mean serum 17β-estradiol data found no significant difference ($P > 0.05$) between hormone implanted and control fish, for any implant month (Appendix 32a).Similar two way ANOVA using $\log_{10}$ transformed mean serum testosterone data found no significant difference ($P > 0.05$) between hormone implanted and control fish, for any implant month (Appendix 32b). However there was a significant ($P < 0.05$) contribution from the interaction between the month of implanting and the implant treatment used. This indicates that mean serum testosterone levels of hormone

treated and control fish were not significantly different and differences which did exist, were not consistent over the different months of the trial.

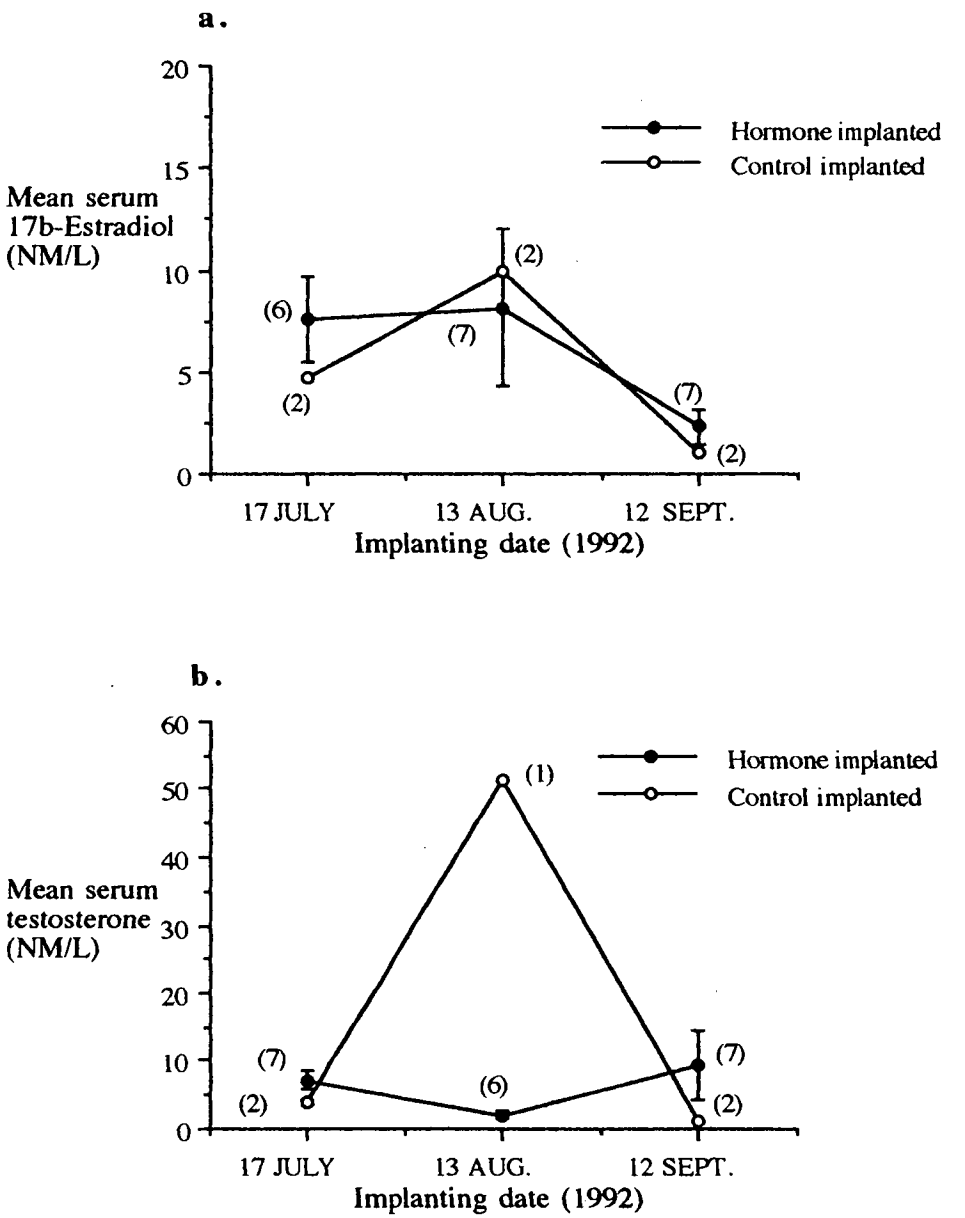


Figure 6.19 Mean serum sex steroid levels (+/- standard error) at each monthly implant, for hormone treated and control *Latris lineata*, during the 1992 UTL sustained release hormone implant trial.

a. 17b-Estradiol **b.** Testosterone

Mean serum 17β-estradiol and testosterone levels of individual females which ovulated eggs during this investigation (implanted fish 304 and 305, and control fish 314 and 315) are presented in Figures 6.20a and 6.20b respectively. In this analysis RIA results of both blood samples taken within the 6 day sampling period, for each monthly implant, are combined for each fish (N=4 samples/treatment/month). Two way ANOVA (Appendix 33a) reveals that mean serum 17β-estradiol levels of hormone implanted and control fish differ significantly ($P< 0.05$) between treatments and implant month. One way ANOVA (Appendix 34) reveals that mean serum 17β-estradiol levels of hormone implanted fish were significantly higher ($P< 0.05$) than control fish, at the time of both the July and August implants.

Mean serum testosterone remained low at each implant month for hormone implanted fish. Two way ANOVA (Appendix 33b) shows no significant ($P> 0.05$) change in mean serum testosterone levels due to implant treatment. However, there was a significant ($P< 0.05$) change in mean serum testosterone level attributed to implant month.

A representation of the times at which each batch of eggs was ovulated is included in Figure 6.20a. In both implanted fish responsible for ovulating multiple batches (304, 305) of eggs, the level of mean serum 17b-estradiol was elevated while the level of mean serum testosterone was low, throughout the duration of this trial. This observation is in agreement with the two-cell model of steroid production by the oocyte follicle (Kagawa *et al*, 1982). The explaination provided by this model is that low levels of serum testosterone during vitellogenesis is expected, due to the role of this steroid as a precursor for GtH stimulated 17β-estradiol synthesis by the follicle. A sharp decline in the level of serum 17β-estradiol is reported to follow the completion of vitelogenesis, and preceed final oocyte maturation in many species of teleosts (section 5.3.3). This decrease is attributed to a shift in the steroidogenic capacity of ovarian follicles from the secretion of predominantly 17β-estradiol, to secretion of

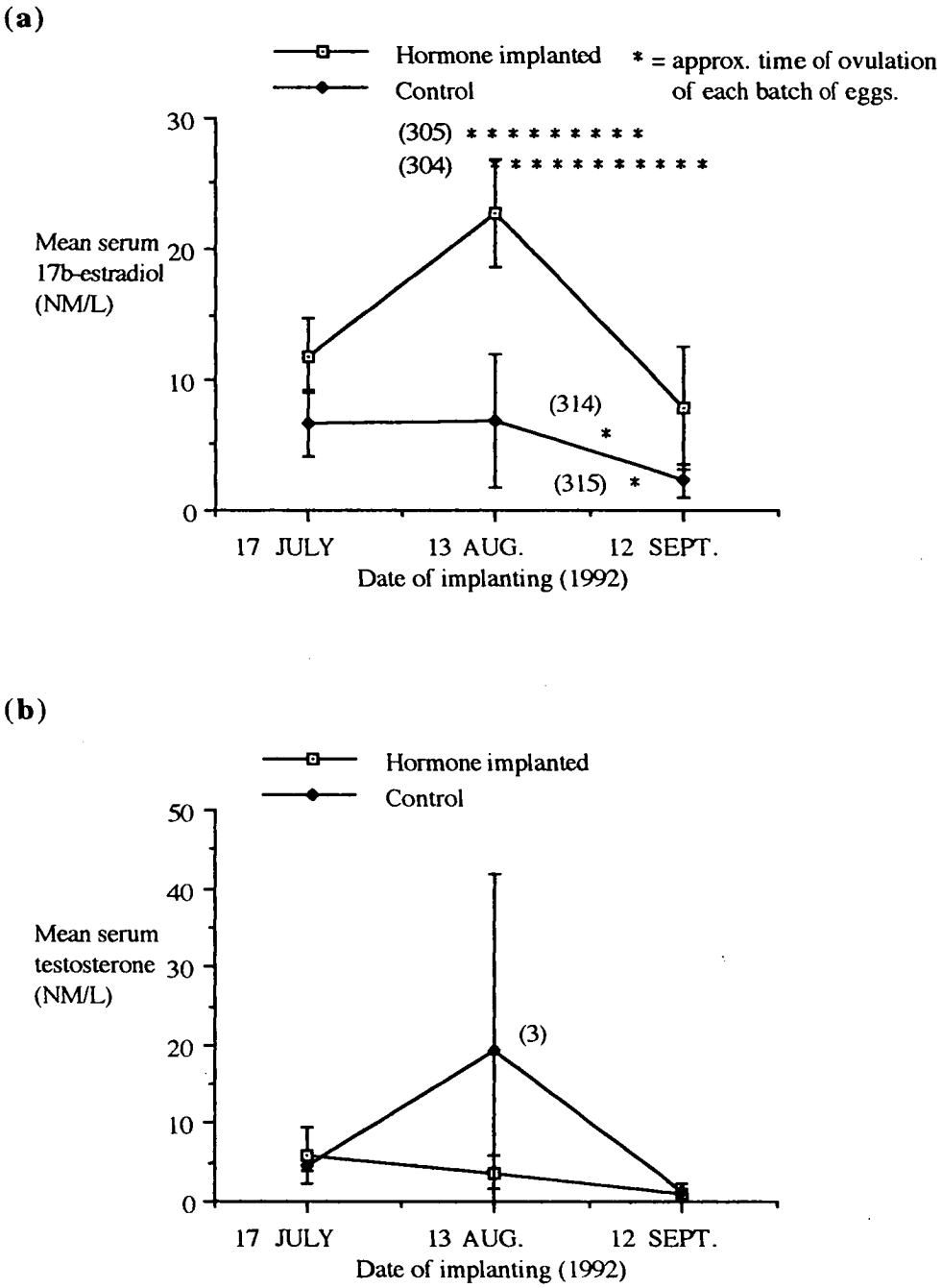


Figure 6.20 Mean serum levels of sex steroids in *Latris lineata* which ovulated eggs during the 1992 UTL sustained release hormone implant trial.

a. 17b-estradiol **b.** testosterone

(Implanted fish = 304, 305; Control fish =314, 315).

n = 4 samples per treatment, for each month unless otherwise indicated (n)

maturation inducing steroids such as 17α, 20β-diOHprog (Sakai *et al.*, 1987; Kanamori *et al.*, 1988a; Suzuki *et al.*, 1989).

However, in the current investigation no sharp decline in 17β-estradiol was suggested by the limited samples taken from hormone implanted fish which ovulated multiple batches of eggs. The elevated levels of 17β-estradiol are most likely attributed to production of this steroid by the following batch of vitellogenic follicles, as is suggested as an explaination for a similar situation reported for goldfish (Kobayashi *et al.*, 1987). A transient decrease in the level of 17β-estradiol following ovulation, lasting for only a few hours, has been reported for carp (Levavi-Zermonsky and Yaron, 1986). In *Latris lineata*, the presence of batches of vitellogenic oocytes undergoing vitellogenesis while a leading batch undergoes maturation and ovulation, was demonstrated in the 1990 UTL spawning induction trial. This situation is anticipated in view of the multiple ovulations recorded for the hormone implanted fish. The pattern of reproduction established for *Latris lineata* in section 4.5 also supports the coexistance of a batch of vitellogenic oocytes responsible for maintaining elevated levels of serum 17β-estradiol in females which ovulate multiple batches of oocytes. In this scenario it is envisaged that in any decrease in the level of 17β-estradiol preceding ovulation may not be dramatic, due to a dampening effect of the following batch of vitellogenic follicles. Any such decrease may exist for a short duration in this scenario.

The changes in the mean serum levels of 17β-estradiol and testosterone, for hormone treated and control fish, at 48 hr intervals following each monthly implanting are shown in Figure 6.21. Two way ANOVA was used to analyse the total change (144 hr level - O hr level) in $\log_{10}$ mean sex steroid levels at 144 hr following implanting of female *Latris lineata* with hormone or placebo pellets, for each month of the trial. In these analyses the control fish (N=2) were compared with the same hormone implanted fish (N=2) sampled at 0 hr and 144hr. Although replication in each instance is sparse the results show no significant change ($P > 0.05$) in mean serum 17β-

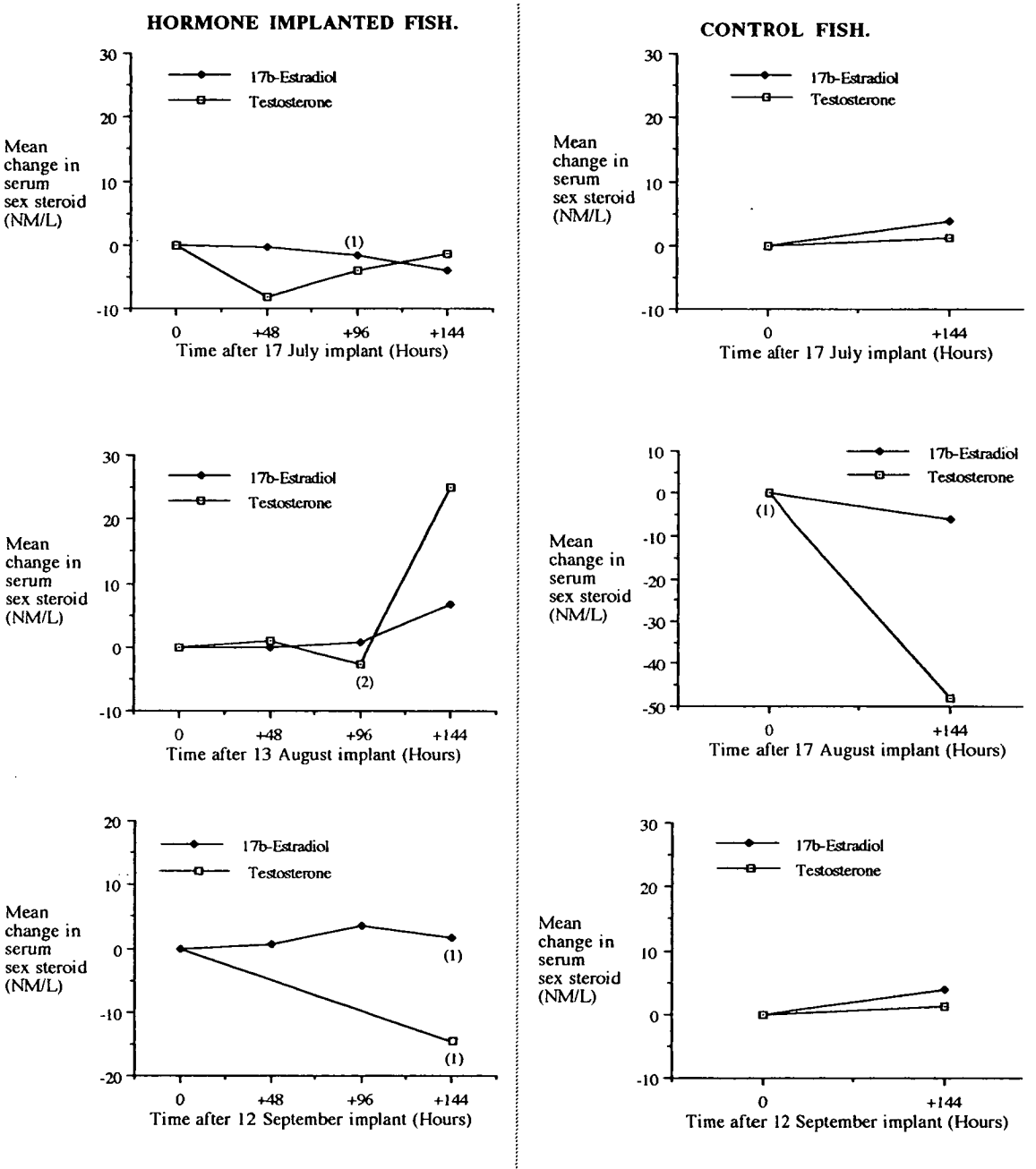


Figure 6.21 Change in mean serum steroid from the initial (0 hr) mean values recorded at each month, for hormone implanted and control *Latris lineata*. N for implanted fish: 0-48 hr = 2; 0-96 hr =3; 0-144 hr =2. N for control fish =2. Unless otherwise indicated (n).

estradiol over the 144 hr interval, between hormone treated and control fish, following any implanting month (Appendices 35a, 35b and 35c.). This result suggests that the hormone implant protocol followed in this trial had no significant effect on the level of mean serum 17β-estradiol level at 6 days following implantation, at any month of implanting.

Analysis of testosterone levels shows that after the 144 hr interval following the July implant, the mean serum testosterone level of control fish (5.19 +/- 0.66 NM/L) was significant greater ($P< 0.05$) than that of hormone implanted fish (2.77 +/- 0.25 NM/L). At this time a significant interaction ($P< 0.05$) between sampling time and experimental treatment was also shown (Appendix 36). A significant ($P< 0.05$) interaction between implant treatment and sampling time was also found in the analysis of mean serum testosterone for the August implant. These results suggests that the mean serum testosterone levels in hormone treated and control fish exhibited opposing responses following implantation at each of these months. This is demonstrated in Figure 6.21 were testosterone levels of hormone treated fish implanted in July fell slightly over the 144 hr period, while a small increase was recorded for control fish. The opposite response followed the August implant, when the mean serum testosterone level of hormone treated fish increased markedly, while that of the control fish fell sharply during the 144 hr period following implantation.

One way ANOVA, followed by comparison of sample means, was conducted to analyse changes from the initial (0 hr) mean sex steroid levels of hormone implanted *Latris lineata* , at 48 hr intervals following each monthly implant. Mean serum 17β-estradiol level was significantly different from the initial level on only one ocassion, at 96 hr following the July implant (Appendix 37).

This comparison could only be conducted for mean serum testosterone following the July and August implants, as 48 hr and 96 hr September samples were not available because initial asays were outside the range of test standards and insufficient serum remained to repeat these assays. Following the July implant a significant ($P > 0.05$) decrease from the initial (0 hr) level of mean serum testosterone was recorded at 48 hours, 96 hr and 144 hr following implantation. However, there was no significant difference ($P > 0.05$) in the mean serum testosterone level recorded between any of the 48 hr sampling intervals (Figure 6.21; Appendix 38). Following the August implant mean serum testosterone had increased significantly ($P < 0.05$) after 144 hr and a significant ($P < 0.05$) increase in this steroid was found to have occurred between 96 hr and 144 hr following this implant (Figure 6.21)

Taken in combination with the analysis of changes in the levels of serum sex steroids during the 24 hr interval following implanting from the DSF investigation, these results indicate there was a significant reduction in the level of serum testosterone in hormone implanted female *Latris lineata* during early gonad recrudescence, at the time of the July implant. The UTL investigation has shown that the level of mean serum testosterone levels of pre-spawning, hormone implanted fish were significantly elevated above initial levels by 144 hr following the implanting procedure, although no significant difference was recorded during this time between these fish, and fish implanted with placebo pellets. Both investigations demonstrated that mean serum 17β-estradiol levels of female *Latris lineata* did not respond to hormone implant treatment within the 144 hr sampling interval encompassed by these trials. No differences between mean serum 17β-estradiol levels of hormone implanted and control fish were recorded at any month or sampling interval following implantation, in either investigation.

These findings suggested that changes in the level of serum testosterone may occur within the 144 hr period covered by these investigations. Changes (if any) in serum

17β-estradiol induced by the sustained release hormone implants used in these investigations may occur outside the 144 hr post-implanting interval covered by the results presented. As previously suggested, the determination of serum GtH levels, in conjunction with sex steroid assays, would assist in the elucidation of the effects of i.m. implanted sustained release hormone pellets in female *Latris lineata*.

Crim *et al.* (1983a) demonstrated that pelleted LHRHa stimulated chronic GtH release in landlocked Atlantic salmon, for periods of up to 4 weeks. In this study a decline in plasma GtH was observed 7 days following implanting of recrudescing females, however elevated levels were recorded at day 21. Implanting of pre-spawning females resulted in a sustained release of GtH inducing ovulation. Figure 6.20a suggests that a sustained release of GtH may be responsible for maintaining the multiple ovulations recorded for two hormone implanted *Latris lineata* in the UTL investigation. Crim *et al.* (1983b) recorded increased circulating GtH in rainbow trout at 2 weeks following LHRHa pellet administration although no samples were taken until this time. In this study, circulating GtH of fish which had received pellets was significantly elevated above that of control fish for a period of 8 weeks. Sokolowska *et al.* (1984) recorded an increase in serum GtH for goldfish after implantation with LHRHa in a cholesterol pellet. The increase in serum GtH was observed 2 days after implantation of females held at 12 - 14 °C, and after 1 day for females held at 18 - 20 °C. Such results support the need to extend the sampling protocol used in this study in any investigations aimed at following the effect of sustained release hormone implants, on the levels of circulating sex steroids and GtH in *Latris lineata*.

7 CONCLUSIONS.

The intention of this study was primarily to provide an understanding of the reproductive biology of wild caught *Latris lineata*, upon which controlled spawning studies could be developed. Although limited by a relatively small number (N = 334) of fish captured, the results presented identify and define the stages of reproductive development for male and female *Latris lineata* which are in accordance with developmental stages identified for other species of teleosts. Such information should prove valuable to the wild fishery and will assist assessment of the state of reproductive development of *Latris lineata* broodstock maintained for controlled spawning purposes.

In this study monitoring of gonad index at different months, and staging of developing ovaries has shown that *Latris lineata* is a spring spawning species which develops batches of oocytes in a group synchronous manner. Analysis of frequency distributions of oocyte diameters in histological sections has identified that during the spawning season three batches of developing oocytes can be distinguished within the ovary of female *Latris lineata*.

The age at first maturity data presented in this study suggests that female *Latris lineata* first mature at 5 years of age (FL = 43.5 cm) while males first mature at 8 years of age (FL = 53.4 cm). The mean age at first maturity for females was 7.0 +/- 1.2 years, while that of males was 9.2 +/- 1.1 years. Considerable overlap exists in these data such that the maturity of most fish cannot be assured until the size exceeds FL=60 cm. This information indicates that *Latris lineata* is slow growing in the environment in which these fish were captured; a finding that may help explain why this species is so susceptible to fishing pressure. The implication of this finding, with respect to aquaculture is that growth rate under culture conditions will need to greatly exceed that achieved by wild caught fish if such operations are to be economically viable.

Spawning induction investigations in 1990 and 1991 provide preliminary results which suggest that induction procedures using either Ovaprim® or LHRHa can be successfully employed for female *Latris lineata* approaching spawning which have completed vitellogenesis and have mean oocyte diameters which exceed approximately 600 μm. This figure has been confirmed in histological studies as the approximate size for the commencement of germinal vesicle migration, final oocyte maturation, ovulation and subsequent hydration.

Results of sustained release hormone implant investigations confirm the group synchronous pattern of oocyte development identified in wild caught fish. Although only 2 female fish responded to the high dose LHRHa + 17α -methyltestosterone monthly pellet implant treatment, these fish ovulated multiple batches of eggs at approximately 3 day intervals. The earliest of these fish ovulated a batch of eggs 21 days in advance of the first ovulation recorded for a control fish. This observation indicates that potential exists to employ sustained release hormone implant procedures to advance the spawning season of captive *Latris lineata.* In addition, the spontaneous release of eggs into the tank recorded for hormone implanted and control fish during this investigation suggests that this situation may be routinely achieved with further refinement of spawning induction procedures.

Sex steriod assays were conducted to monitor the effect of hormone implant treatments on circulating levels of 17β-estradiol and testosterone at various intervals up to 144 hr after implantation. Results of these assays were largely inconclusive as a large amount of variation was recorded in these data. This variability was compounded by an over ambitious experimental design which resulted in a small number of treatment replicates. Replication was restricted in all experiments by the capacity of facilities to hold substantial numbers of broodstock. Variability was also compounded by the wide range of stages of development present in the limited number of female fish available for each investigation.

Although no statistically significant differences ($P > 0.05$) in mean serum 17β-estradiol and testosterone between hormone implant treatments have been found in the 1992 DSF investigation, apparent trends and results from the most responsive fish are in agreement with results expected if the two-cell model for steroid production by the oocyte follicle (Kagawa *et al.*, 1982) is accepted. This was also the case in the 1992 UTL experiment. These investigations both suggest that any future investigations should be commenced with a group of mature females with a uniform state of oocyte development.

In both investigations no significant ($P > 0.05$) changes in levels of mean serum 17β-estradiol could be attributed to the sustained release hormone implants over a 0 - 144hr sampling interval. In the 1992 DSF investigation, mean serum testosterone decreased at 24 hr after the July implant in fish receiving the high dose pellet treatment, while an increase was recorded for control fish. A similar situation was observed at 144 hr following the July implant in the 1992 UTL investigation were mean serum testosterone levels of hormone implanted fish were significantly ($P < 0.05$) lower than those of control fish at this time. In this investigation mean serum testosterone levels of hormone implanted females increased significantly between 96 and 144 hr after the August implant. In the 1992 UTL investigation the two females which ovulated batches of eggs had significantly ($P < 0.05$) elevated levels of 17β-estradiol at the time of the July and August implants, while the level of serum testosterone was low at both of these occasions.

In all of the hormone induction studies presented the effect of holding environment cannot be ignored. The results obtained during investigations at UTL and at DSF in 1991 are likely to have been adversely effected by the relatively small volume of the holding tanks available (4 m3). Large tank size is identified as a positive contributing factor in the success of egg production from captive broodstock and most researchers employ large tank sizes for broodstock (Harvey *et al.*, 1985; Garrett and Rasmussen,

1987; Zohar *et al.*, 1989). This effect will be significant with a large species such as *Latris lineata*. Other negative handling, transport and changes in holding facilities (outdoor tank initially, then into a sea cage then back into the outdoor tank) and water quality (temperature, salinity) accompanied the 1992 DSF hormone implant investigation. Unfortunately these changes were imposed due to the commitment of facilities to higher priority needs. However, undoubtedly these changes would have exposed experimental broodfish to considerable stressors which have been identified as reducing the success of spawning induction procedures (Billard *et al.*, 1981).

It must also be noted that photoperiod and water temperature were both carefully controlled in the 1992 UTL investigation which achieved successful induction of spawning, and ultimately successful larval rearing of *Latris lineata*. As previously described, both of these environmental parameters have been identified as being controlling factors in the reproductive development of teleosts (Billard *et al.*, 1981; Lam, 1983; Hanyu and Razani, 1985). It is suggested that control over these parameters provided a more uniform holding environment, which together with reduced handling stress, could only have assisted the achievement of the results achieved in this investigation.

The results of this study supports the continuation of research into the controlled spawning of *Latris lineata*. In particular, the successful induction of multiple batches of eggs and the advancement of the spawning period achieved during the 1992 UTL investigation suggests that more consistent research outcomes could be achieved with dedicated facilities specifically designed to provide a controlled (photoperiod, water temperature) environment in which broodstock can be maintained and handled in a manner which minimises stress. Ideally these facilities would need to be large in order to promote successful spawning and to accommodate the numbers of broodstock needed to provide the replication required to reduce experimental variability in any future investigations conducted on a large marine species such as *Latris lineata*.

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APPENDICES

APPENDIX 1. Biological data for wild-caught striped trumpeter, Latris lineata.

SAMPLING DATE	SEX	FORK LTH. (cm)	TOTAL WET WEIGHT (g).	CONDITION FACTOR	GUT LTH. (cm)	WEIGHT GUT (g)	REL. GUT LTH.	% GUT WEIGHT
May 25, 1990	MALE	58.5	2400	1.20	62.0	242.7	1.06	10.11
May 25, 1990	MALE	66.0	3700	1.29	73.0	426.4	1.11	11.52
May 25, 1990	MALE	60.0	2700	1.25	77.0	309.0	1.28	11.44
May 25, 1990	MALE	53.0	2200	1.48	62.0	219.1	1.17	9.96
May 25, 1990	MALE	63.5	3300	1.29	78.0	299.0	1.23	9.06
May 25, 1990	MALE	54.0	2200	1.40	63.0	196.1	1.17	8.91
May 25, 1990	MALE	69.0	4200	1.28	64.0	440.5	0.93	10.49
May 25, 1990	MALE	60.0	2600	1.20	62.0	255.4	1.03	9.82
May 25, 1990	MALE	55.5	2200	1.29	62.0	206.3	1.12	9.38
May 25, 1990	MALE	66.0	4500	1.57	55.0	451.7	0.83	10.04
May 25, 1990	FEMALE	53.5	2100	1.37	64.0	198.7	1.20	9.46
May 25, 1990	FEMALE	49.0	1500	1.27	49.0	144.1	1.00	9.61
May 25, 1990	FEMALE	49.0	1400	1.19	43.0	151.1	0.88	10.79
May 25, 1990	FEMALE	61.0	2600	1.15	70.0	291.1	1.15	11.20
May 25, 1990	FEMALE	61.0	3100	1.37	70.0	33.1.5	1.15	10.69
May 25, 1990	FEMALE	80.0	7500	1.46	86.0	708.7	1.08	9.45
May 25, 1990	FEMALE	59.5	2400	1.14	69.0	276.7	1.16	11.53
May 25, 1990	FEMALE	71.0	5500	1.54	92.0	588.9	1.30	10.71
May 25, 1990	FEMALE	59.5	2600	1.23	68.0	334.9	1.14	12.88
May 25, 1990	FEMALE	49.5	1500	1.24	49.0	175.7	0.99	11.71
Jul 12, 1990	MALE	75.0	5560	1.32				
Jul 12, 1990	MALE	51.0	1820	1.37				
Jul 12, 1990	MALE	58.0	2660	1.36				
Jul 12, 1990	MALE	54.5	2200	1.36				
Jul 12, 1990	MALE	56.0	2580	1.47				
Jul 12, 1990	MALE	66.5	4000	1.36				
Jul 12, 1990	MALE	51.5	1760	1.29				
Jul 12, 1990	MALE	64.0	3300	1.26				
Jul 12, 1990	MALE	54.5	2100	1.30				
Jul 12, 1990	MALE	58.5	2640	1.32				
Jul 12, 1990	MALE	62.5	3200	1.31				
Jul 12, 1990	MALE	54.5	2000	1.24				
Jul 12, 1990	FEMALE	61.0	2760	1.22				
Jul 12, 1990	FEMALE	60.0	2860	1.32				
Jul 12, 1990	FEMALE	60.0	2580	1.19				
Jul 12, 1990	FEMALE	62.0	3100	1.30				
Jul 12, 1990	FEMALE	63.0	2900	1.16				
Jul 12, 1990	FEMALE	65.0	3640	1.33				
Jul 12, 1990	FEMALE	55.0	2330	1.40				
Jul 12, 1990	FEMALE	62.0	3040	1.28				
Jul 12, 1990	FEMALE	59.0	2750	1.34				
Jul 12, 1990	FEMALE	55.5	2700	1.58				
Jul 12, 1990	FEMALE	66.5	3860	1.31				
Jul 12, 1990	FEMALE	82.0	7880	1.43				
Jul 12, 1990	FEMALE	65.0	3140	1.14				
Oct 4, 1991	MALE	59.5	3050	1.45				
Oct 4, 1991	MALE	66.5	4200	1.43				
Oct 4, 1991	MALE	58.5	2820	1.41				
Oct 4, 1991	MALE	60.0	3450	1.60				
Oct 4, 1991	MALE	56.5	2700	1.50				
Oct 4, 1991	MALE	58.5	2940	1.47				
Oct 4, 1991	MALE	62.0	3300	1.38				
Oct 4, 1991	MALE	68.0	4720	1.50				
Oct 4, 1991	FEMALE	51.5	2000	1.46				
Oct 4, 1991	FEMALE	83.0	9400	1.64				
Oct 4, 1991	FEMALE	64.5	4180	1.56				
Oct 4, 1991	FEMALE	54.0	2120	1.35				
Oct 4, 1991	FEMALE	54.5	2520	1.56				
Oct 4, 1991	FEMALE	54.5	2360	1.46				
Oct 4, 1991	FEMALE	65.5	3800	1.35				
Oct 4, 1991	FEMALE	54.0	2250	1.43				
Oct 4, 1991	FEMALE	78.5	7900	1.63				
Oct 4, 1991	FEMALE	56.0	2460	1.40				
Oct 4, 1991	FEMALE	62.0	3260	1.37				
Oct 4, 1991	FEMALE	67.5	4560	1.48				

APPENDIX 2. Statistical analysis of the relationship between the dependant variables month and sex and the independant variables fork length (cm), wet weight (g) and condition factor (100 x weight / fork length^3) of *Latris lineata*. (P = 0.05)

a. ANOVA table for comparison of sex, month and fork length (cm).

Anova table for a 2-factor Analysis of Variance on Y_1: FORK LTH.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
SEX (A)	1	22.562	22.562	.377	.5414
MONTH (B)	2	29.397	14.699	.246	.7829
AB	2	74.959	37.479	.627	.5379
Error	59	3528.726	59.809		

There were no missing cells found.

b. ANOVA table for comparison of sex, month and total fish weight (g).

Anova table for a 2-factor Analysis of Variance on Y_2: WEIGHT

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
SEX (A)	1	1954665.077	1954665.077	.791	.3774
MONTH (B)	2	4875090.319	2437545.16	.986	.379
AB	2	858673.86	429336.93	.174	.841
Error	59	145808900.641	2471337.299		

There were no missing cells found.

c. ANOVA table for comparison of sex, month and condition factor.

Anova table for a 2-factor Analysis of Variance on Y_1: arcsin(x) of 1/x of COND. FACTOR

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
SEX (A)	1	.007	.007	.947	.3346
MONTH (B)	2	.213	.106	13.983	.0001
AB	2	.004	.002	.244	.7843
Error	59	.449	.008		

There were no missing cells found.

APPENDIX 3. Statistical analysis of month and condition factor data for *Latris lineata* (P = 0.05).

a. ANOVA table for comparison of month and condition factor

One Factor ANOVA X_1: MONTH Y_1: arcsin(x) of 1/x of COND. FACTOR

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	2	.22	.11	14.818
Within groups	62	.46	.007	p = .0001
Total	64	.681		

Model II estimate of between component variance = .051

b. Monthly condition factor statistics.

One Factor ANOVA X_1: MONTH Y_1: arcsin(x) of 1/x of COND. FACTOR

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
MAY	20	.885	.11	.025
JUL.	25	.871	.085	.017
OCT.	20	.752	.055	.012

c. Comparison of monthly means for condition factor.

One Factor ANOVA X_1: MONTH Y_1: arcsin(x) of 1/x of COND. FACTOR

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
MAY	20	.885	.11	.025
JUL.	25	.871	.085	.017
OCT.	20	.752	.055	.012

APPENDIX 4. Age of Latris lineata determined from otolith samples.

SAMPLE NO.	SAMPLING DATE	OTOLITH BANDS	AGE (Years).	FORK LTH. (cm).	Log10 F.L.
1	Aug 2, 1991	7	7.8	54.0	1.7324
2	Aug 2, 1991	7	7.8	52.5	1.7202
3	Aug 2, 1991	15	15.8	68.0	1.8325
4	Aug 2, 1991	6	6.8	49.5	1.6946
5	Aug 2, 1991	9	9.8	58.0	1.7634
6	Aug 2, 1991	8	8.8	52.0	1.7160
7	Aug 2, 1991	8	8.8	56.0	1.7482
8	Aug 2, 1991	19	19.8	72.0	1.8573
9	Aug 2, 1991	6	6.8	51.0	1.7076
10	Aug 2, 1991	6	6.8	50.0	1.6990
11	Aug 2, 1991	12	12.8	61.0	1.7853
12	Aug 2, 1991	8	8.8	58.0	1.7634
13	Aug 2, 1991	8	8.8	54.0	1.7324
14	Aug 2, 1991	7	7.8	58.5	1.7672
15	Aug 2, 1991	6	6.8	52.5	1.7202
16	Aug 2, 1991	13	13.8	64.5	1.8096
17	Aug 2, 1991	7	7.8	53.5	1.7284
18	Aug 2, 1991	11	11.8	63.5	1.8028
19	Sep 18, 1991	7	7.9	52.0	1.7160
20	Sep 18, 1991	6	6.9	49.0	1.6902
21	Sep 18, 1991	6	6.9	53.5	1.7284
22	Sep 18, 1991	6	6.9	50.0	1.6990
23	Sep 18, 1991	6	6.9	51.0	1.7076
24	Sep 18, 1991	6	6.9	55.0	1.7404
25	Sep 18, 1991	6	6.9	51.5	1.7118
26	Sep 18, 1991	5	5.9	45.0	1.6532
28	Sep 18, 1991	6	6.9	49.5	1.6946
30	Sep 18, 1991	8	8.9	58.0	1.7634
31	May 28, 1991	6	6.7	53.5	1.7284
32	May 28, 1991	5	5.7	43.5	1.6385
33	May 28, 1991	9	9.7	58.5	1.7672
34	May 28, 1991	8	8.7	56.0	1.7482
35	May 28, 1991	9	9.7	55.0	1.7404
36	May 28, 1991	8	8.7	54.0	1.7324
37	May 28, 1991	7	7.7	54.0	1.7324
38	May 28, 1991	12	12.7	60.5	1.7818
39	May 28, 1991	12	12.7	60.5	1.7818
40	May 28, 1991	10	10.7	61.0	1.7853
41	May 28, 1991	18	18.7	67.0	1.8261
42	May 28, 1991	20	20.7	78.0	1.8921
43	May 28, 1991	19	19.7	77.0	1.8865
44	May 28, 1991	6	6.7	49.0	1.6902
45	May 28, 1991	6	6.7	50.5	1.7033
46	May 28, 1991	6	6.7	52.0	1.7160
47	May 28, 1991	16	16.7	75.0	1.8751
48	Jun 14, 1991	6	6.7	53.5	1.7284
49	Jun 14, 1991	6	6.7	53.5	1.7284
50	Jun 14, 1991	6	6.7	50.5	1.7033

APPENDIX 5. Reproductive data from wild-caught striped trumpeter, Latris lineata.

DATE	SAMPLE NUMBER	SEX	FORK LTH (cm)	GONAD WT.(g)	GONAD INDEX	MAX.DEV. STAGE	MEAN MAX. 10 OOCYTES	S.D. (+/-)	ATRETIC OOCYTES
May 25, 1990	2	F	59.5	13.5	6.41	PN	115.7	2.98	Y
May 25, 1990	6	F	80.0	83.9	16.39	PN	142.4	8.38	Y
May 25, 1990	8	F	61.0	30.3	13.35	PN	136.3	6.00	Y
May 25, 1990	10	F	49.5	6.4	5.28	PN	131.7	10.60	N
May 25, 1990	11	F	59.5	16.5	7.83	PN	126.9	8.70	Y
May 25, 1990	14	F	71.0	46.4	12.96	PN	139.6	13.00	Y
May 25, 1990	16	F	61.0	24.4	10.75	PN	123.8	6.36	Y
May 25, 1990	17	F	49.0	5.5	4.67	PN	104.1	5.90	N
May 25, 1990	18	F	49.0	7.7	6.54	PN	113.5	4.85	N
May 25, 1990	20	F	53.5	9.2	6.01	PN	104.6	4.17	Y
May 25, 1990	1	M	66.0	14.7	5.11	M1			
May 25, 1990	3	M	60.0	5.2	2.41	DV			
May 25, 1990	4	M	69.0	11.6	3.53	SR			
May 25, 1990	5	M	55.5	4.1	2.40	M1			
May 25, 1990	7	M	66.0	10.0	3.48	M1			
May 25, 1990	9	M	58.5	2.1	1.05	DV			
May 25, 1990	12	M	60.0	5.5	2.55	SR			
May 25, 1990	13	M	63.5	3.4	1.33	SR			
May 25, 1990	15	M	53.0	2.7	1.81	M1			
May 25, 1990	19	M	54.0	0.6	0.38	I			
Jul 12, 1990	22	F	65.0	37.4	13.62	CA	261.1	19.54	Y
Jul 12, 1990	23	F	82.0	95.5	17.32	CA	262.5	12.54	Y
Jul 12, 1990	24	F	62.0	32.9	13.80	CA	304.0	12.41	Y
Jul 12, 1990	26	F	61.0	39.5	17.40	CA	293.1	16.49	Y
Jul 12, 1990	27	F	59.0	25.1	12.22	CA	220.2	14.64	Y
Jul 12, 1990	30	F	62.0	43.1	18.08	CA	218.7	15.36	Y
Jul 12, 1990	32	F	65.0	36.2	13.18	CA	266.1	23.56	Y
Jul 12, 1990	33	F	63.0	25.0	10.00	PN	163.8	23.02	Y
Jul 12, 1990	37	F	66.5	46.0	15.64	CA	263.8	12.68	Y
Jul 12, 1990	42	F	55.0	16.4	9.86	CA	299.7	16.50	N
Jul 12, 1990	43	F	60.0	31.9	14.77	CA	250.5	17.30	Y
Jul 12, 1990	44	F	60.0	26.9	12.45	PN	158.2	7.16	Y
Jul 12, 1990	21	M	64.0	88.4	33.72	M			
Jul 12, 1990	25	M	51.5	17.3	12.67	M2			
Jul 12, 1990	28	M	51.0	34.7	26.16	M2			
Jul 12, 1990	29	M	75.0	28.2	6.68	M2			
Jul 12, 1990	31	M	58.5	103.9	51.90	M			
Jul 12, 1990	34	M	54.5	3.4	2.10	DV			
Jul 12, 1990	35	M	66.5	72.1	24.52	M2			
Jul 12, 1990	36	M	58.0	54.4	27.88	M2			
Jul 12, 1990	38	M	62.5	90.3	36.99				
Jul 12, 1990	39	M	56.0	1.4	0.80	DV			
Jul 12, 1990	40	M	55.5	35.5	20.77				
Jul 12, 1990	41	M	54.5	15.5	9.58				
Jul 12, 1990	45	M	54.5	21.5	13.28				
Sep 20, 1990	1	F	58.5	104.5	52.20	GVM	579.6	30.05	
Sep 20, 1990	2	F	62.0	10.8	4.53	CA	290.5	25.66	
Sep 20, 1990	4	F	58.5	16.1	8.03	CA	293.1	13.54	
Sep 20, 1990	7	F	54.0	13.6	8.65	CA	311.6	15.85	
Sep 20, 1990	17	F	58.5	72.3	36.11	GVM	665.4	41.37	
Sep 20, 1990	18	F	56.5	9.8	5.43	CA	302.1	29.93	
Sep 20, 1990	20	F	61.0	35.9	15.82	CA			
Sep 20, 1990	22	F	56.0	65.1	37.07	GVM	614.7	23.89	
Sep 20, 1990	24	F	54.5	54.4	33.61	GVM	681.8	25.46	
Sep 20, 1990	25	F	55.5	7.8	4.56	CA	226.5	9.55	
Sep 20, 1990	26	F	56.5	63.0	34.93	GVM	647.5	31.28	
Sep 20, 1990	27	F	55.5	69.2	40.48	VC	652.3	32.04	
Sep 20, 1990	29	F	54.0	45.0	28.58	VC	587.6	85.22	
Sep 20, 1990	30	F	56.0	8.7	4.95	CA	211.2	19.36	
Sep 20, 1990	35	F	57.0	14.9	8.05				
Sep 20, 1990	36	F	70.5	144.5	41.24	VC	635.5	15.05	
Sep 20, 1990	38	F	54.0	9.3	5.91	CA	305.4	20.07	

APPENDIX 5. Reproductive data from wild-caught striped trumpeter, Latris lineata.

DATE	SAMPLE NUMBER	SEX	FORK LTH (cm)	GONAD WT.(g)	GONAD INDEX	MAX.DEV. STAGE	MEAN MAX. 10 OOCYTES	S.D. (+/-)	ATRETIC OOCYTES
Sep 20, 1990	40	F	56.0	10.1	5.75	PN	178.9	8.14	
Sep 20, 1990	21	M	56.0	82.8	47.15				
Sep 20, 1990	23	M	52.5	30.4	21.02				
Sep 20, 1990	31	M	61.5	44.1	18.97	M2			
Sep 20, 1990	32	M	56.0	91.0	51.79				
Sep 20, 1990	33	M	58.0	104.1	53.36	R			
Sep 20, 1990	37	M	59.5	182.8	86.78	R			
Sep 20, 1990	39	M	51.0	12.9	9.69	M1			
Sep 29, 1990	2	F	63.0	132.7	53.07	GVM	638.7	15.13	
Sep 29, 1990	6	F	52.5	79.5	54.94	GVM	700.6	20.55	
Sep 29, 1990	7	F	68.0	211.9	67.39	GVM	593.3	12.40	
Sep 29, 1990	8	F	84.0	501.4	84.60	GVM	598.8	27.28	
Sep 29, 1990	9	F	70.0	388.8	113.35				
Sep 29, 1990	10	F	50.0	36.4	29.12	MAT	860.0	26.05	
Sep 29, 1990	11	F	60.5	87.9	39.69	GVM	602.1	16.09	
Sep 29, 1990	12	F	51.0	68.4	51.56	GVM	629.1	27.14	
Sep 29, 1990	17	F	66.0	111.7	38.85	GVM	622.9	16.42	
Sep 29, 1990	20	F	52.0	77.4	55.05	MAT	815.4	16.12	
Sep 29, 1990	22	F	65.0	134.3	48.90	GVM	623.4	29.47	
Sep 29, 1990	29	F	56.0	69.8	39.75	GVM	557.6	26.47	
Sep 29, 1990	30	F	72.5	272.8	71.59	GVM	590.7	30.54	
Sep 29, 1990	31	F	53.0	38.4	25.79	GVM	573.5	25.87	
Sep 29, 1990	32	F	78.5	443.3	91.64	GVM	651.4	26.78	
Sep 29, 1990	39	F	65.5	150.8	53.66	NT	584.2	12.61	
Sep 29, 1990	42	F	52.0	66.2	47.08	MAT	872.7	9.55	
Sep 29, 1990	1	M	62.0	98.2	41.20				
Sep 29, 1990	3	M	59.0	133.8	65.15	R			
Sep 29, 1990	4	M	58.0	77.1	39.52				
Sep 29, 1990	5	M	61.5	169.9	73.04	R			
Sep 29, 1990	13	M	52.0	57.7	41.04				
Sep 29, 1990	14	M	58.5	1.2	0.60	I			
Sep 29, 1990	15	M	58.5	69.7	34.81				
Sep 29, 1990	16	M	54.5	75.3	46.52				
Sep 29, 1990	18	M	58.0	101.3	51.92	R			
Sep 29, 1990	19	M	52.0	114.8	81.65				
Sep 29, 1990	21	M	53.0	21.1	14.17	M2			
Sep 29, 1990	23	M	54.0	113.5	72.08				
Sep 29, 1990	24	M	56.5	45.7	25.34				
Sep 29, 1990	25	M	54.0	57.0	36.20				
Sep 29, 1990	26	M	55.0	55.4	33.30				
Sep 29, 1990	27	M	57.0	43.9	23.70				
Sep 29, 1990	28	M	63.5	151.9	59.32				
Sep 29, 1990	33	M	56.5	131.3	72.80				
Sep 29, 1990	34	M	50.0	98.2	78.56				
Sep 29, 1990	35	M	51.5	9.7	7.10				
Sep 29, 1990	36	M	50.5	22.3	17.32				
Sep 29, 1990	37	M	67.5	295.0	95.92				
Sep 29, 1990	38	M	50.0	104.5	83.60				
Sep 29, 1990	40	M	62.5	82.6	33.83				
Sep 29, 1990	41	M	59.0	107.5	52.34	M			
Sep 29, 1990	43	M	58.0	17.8	9.12				
Sep 29, 1990	44	M	56.0	128.5	73.17				
Sep 29, 1990	45	M	58.0	105.9	54.28				
Mar 14, 1991	2	F	77.0	87.6	19.19				
Mar 14, 1991	3	F	72.0	48.4	12.97	PN	129.1	5.53	Y
Mar 14, 1991	7	F	55.5	11.5	6.73	PN	125.8	10.21	N
Mar 14, 1991	9	F	55.0	10.2	6.13	PN	125.7	7.10	Y
Mar 14, 1991	11	F	49.5	8.5	7.01	PN	117.3	3.74	N
Mar 14, 1991	12	F	55.0	11.7	7.03	PN	121.0	7.17	N
Mar 14, 1991	18	F	68.5	51.2	15.93	PN	128.7	7.50	Y
Mar 14, 1991	23	F	52.5	10.8	7.46	PN	123.3	4.17	Y
Mar 14, 1991	26	F	49.0	7.6	6.46	PN	129.1	6.97	N

APPENDIX 5. Reproductive data from wild-caught striped trumpeter, Latris lineata.

DATE	SAMPLE NUMBER	SEX	FORK LTH (cm)	GONAD WT.(g)	GONAD INDEX	MAX.DEV. STAGE	MEAN MAX. 10 OOCYTES	S.D. (+/-)	ATRETIC OOCYTES
Mar 14, 1991	30	F	52.0	12.1	8.61				N
Mar 14, 1991	31	F	52.5	8.0	5.53				
Mar 14, 1991	32	F	56.0	9.5	5.41				
Mar 14, 1991	34	F	48.5	8.2	7.19	PN	131.2	3.62	N
Mar 14, 1991	36	F	84.0	83.1	14.02		129.1	4.33	
Mar 14, 1991	37	F	59.5	30.2	14.34	PN			Y
Mar 14, 1991	39	F	74.0	42.7	10.54	PN	119.7	6.63	Y
Mar 14, 1991	40	F	65.0	32.2	11.73				
Mar 14, 1991	41	F	70.5	34.3	9.79				
Mar 14, 1991	42	F	56.0	8.5	4.84	PN	129.5	4.53	N
Mar 14, 1991	43	F	64.0	21.9	8.35				
Mar 14, 1991	44	F	63.0	33.1	13.24				
Mar 14, 1991	45	F	83.0	80.0	13.99	PN	106.1	10.82	Y
Mar 14, 1991	46	F	70.5	50.3	14.35				
Mar 14, 1991	48	F	63.0	32.1	12.84				
Mar 14, 1991	49	F	54.0	10.3	6.54				
Mar 14, 1991	53	F	61.5	25.8	11.09				
Mar 14, 1991	54	F	66.0	42.3	14.71				
Mar 14, 1991	1	M	64.0	10.1	3.85	SR			
Mar 14, 1991	4	M	64.0	10.6	4.04	SR			
Mar 14, 1991	6	M	50.0	0.6	0.48	I			
Mar 14, 1991	8	M	65.5	10.7	3.81	SR			
Mar 14, 1991	10	M	64.0	10.8	4.12	SR			
Mar 14, 1991	14	M	52.0	2.0	1.42	DV			
Mar 14, 1991	15	M	64.0	7.8	2.98	SR			
Mar 14, 1991	16	M	55.0	2.6	1.56				
Mar 14, 1991	17	M	71.0	18.1	5.06				
Mar 14, 1991	19	M	53.0	0.8	0.54				
Mar 14, 1991	20	M	61.0	2.9	1.28	DV			
Mar 14, 1991	21	M	53.0	2.1	1.41	SR			
Mar 14, 1991	22	M	52.5	3.2	2.21				
Mar 14, 1991	24	M	56.5	4.7	2.61	SR			
Mar 14, 1991	25	M	58.0	4.3	2.20				
Mar 14, 1991	27	M	55.0	2.2	1.32	DV			
Mar 14, 1991	28	M	52.5	2.1	1.45				
Mar 14, 1991	29	M	54.5	4.1	2.53				
Mar 14, 1991	33	M	55.0	1.1	0.66	DV			
Mar 14, 1991	35	M	69.5	12.2	3.63				
Mar 14, 1991	38	M	71.0	9.6	2.68				
Mar 14, 1991	47	M	65.0	15.6	5.68				
Mar 14, 1991	50	M	53.0	1.6	1.07				
Mar 14, 1991	51	M	49.5	0.6	0.49				
Mar 14, 1991	52	M	72.0	11.6	3.11				
Jun 14, 1991	2	F	56.5	15.8	8.77	PN	132.7	5.66	N
Jun 14, 1991	3	F	50.5	10.1	7.84	PN	141.7	6.65	N
Jun 14, 1991	5	F	54.5	10.8	6.70	PN	130.9	4.93	N
Jun 14, 1991	6	F	50.0	9.1	7.24	PN	124.2	8.36	N
Jun 14, 1991	8	F	50.0	7.4	5.90	PN	122.7	4.20	N
Jun 14, 1991	9	F	76.0	74.5	16.96	PN	168.1	16.21	Y
Jun 14, 1991	10	F	65.5	52.2	18.58	PN	148.6	6.24	Y
Jun 14, 1991	13	F	52.5	12.6	8.67	CA	178.0	4.81	N
Jun 14, 1991	16	F	47.5	6.1	5.71	PN	158.9	10.34	N
Jun 14, 1991	17	F	53.5	11.9	7.78	PN	166.1	7.22	N
Jun 14, 1991	18	F	53.0	7.2	4.86	PN	129.5	2.55	N
Jun 14, 1991	19	F	52.0	9.8	6.99	CA	163.9	4.75	N
Jun 14, 1991	1	M	65.0	16.5	6.01	M1			
Jun 14, 1991	4	M	50.0	1.9	1.55	M1			
Jun 14, 1991	7	M	60.0	9.0	4.17	M1			
Jun 14, 1991	11	M	57.0	7.5	4.06	M1			
Jun 14, 1991	12	M	53.5	1.7	1.10	SR			
Jun 14, 1991	14	M	46.0	0.4	0.38	I			
Jun 14, 1991	15	M	58.5	4.3	2.17	SR			

APPENDIX 5. Reproductive data from wild-caught striped trumpeter, Latris lineata.

DATE	SAMPLE NUMBER	SEX	FORK LTH (cm)	GONAD WT.(g)	GONAD INDEX	MAX.DEV. STAGE	MEAN MAX. 10 OOCYTES	S.D. (+/-)	ATRETIC OOCYTES
Jun 14, 1991	20	M	53.0	3.6	2.40	M1			
Jun 14, 1991	21	M	61.0	5.6	2.47	SR			
Oct 4, 1991	37	F	62.0	125.9	52.83				
Oct 4, 1991	38	F	65.5	51.3	18.26	VC	564.7	27.70	
Oct 4, 1991	40	F	78.5	459.7	95.03	GVM	627.4	24.40	
Oct 4, 1991	46	F	56.0	87.7	49.94	VC	566.7	9.02	
Oct 4, 1991	47	F	54.5	22.5	13.90	NT	138.4	4.43	
Oct 4, 1991	49	F	51.5	17.1	12.52	NT	129.2	4.85	
Oct 4, 1991	33	F	54.5		0.00				
Oct 4, 1991	35	F	64.5	152.4	56.79				
Oct 4, 1991	36	F	83.0	435.8	76.22				
Oct 4, 1991	39	F	54.0	180.4	114.57				
Oct 4, 1991	43	F	67.5	161.5	52.51				
Oct 4, 1991	52	F	54.0	19.2	12.19				
Oct 4, 1991	41	M	58.5	135.9	67.88	R			
Oct 4, 1991	48	M	60.0	88.4	40.93	R			
Oct 4, 1991	50	M	62.0	149.2	62.60	R			
Oct 4, 1991	51	M	58.5	122.4	61.14	R			
Oct 4, 1991	53	M	56.5	124.6	69.08	R			
Oct 4, 1991	34	M	68.0		0.00				
Oct 4, 1991	42	M	66.5	289.8	98.54				
Oct 4, 1991	45	M	59.5	154.9	73.54				
Jan 22, 1992	4	F	60.0	19.9	9.19	PN	123.6	9.57	Y
Jan 22, 1992	5	F	58.5	24.2	12.06				
Jan 22, 1992	6	F	53.5	11.0	7.19	PN	122.1	8.50	N
Jan 22, 1992	7	F	56.5	20.0	11.11	PN	115.7	3.80	N
Jan 22, 1992	8	F	57.0	19.3	10.41	PN	117.8	9.41	Y
Jan 22, 1992	11	F	51.0	11.2	8.41				
Jan 22, 1992	12	F	52.0	13.5	9.61				
Jan 22, 1992	14	F	43.5	2.4	2.92	PN	95.8	5.59	N
Jan 22, 1992	16	F	54.0	12.5	7.91				
Jan 22, 1992	18	F	55.5	19.1	11.17				
Jan 22, 1992	19	F	58.0	30.4	15.59				
Jan 22, 1992	21	F	52.5	14.7	10.12				
Jan 22, 1992	22	F	66.0	36.1	12.57	PN	117.1	5.78	Y
Jan 22, 1992	23	F	56.0	13.4	7.62	PN	130.4	6.04	Y
Jan 22, 1992	24	F	66.0	44.7	15.53				
Jan 22, 1992	25	F	49.0	10.5	8.93				
Jan 22, 1992	29	F	61.0	31.3	13.80	PN	107.3	4.50	Y
Jan 22, 1992	32	F	53.5	14.0	9.16				
Jan 22, 1992	35	F	52.0	14.1	9.99				
Jan 22, 1992	36	F	54.0	22.6	14.38	PN	119.1	4.95	Y
Jan 22, 1992	39	F	58.0	26.6	13.61				
Jan 22, 1992	40	F	55.0	10.9	6.53				
Jan 22, 1992	42	F	55.0	15.5	9.29				
Jan 22, 1992	43	F	58.0	17.7	9.07	PN	117.0	4.62	Y
Jan 22, 1992	45	F	52.0	8.1	5.78				
Jan 22, 1992	46	F	69.0	40.8	12.41				
Jan 22, 1992	47	F	64.0	27.0	10.29				
Jan 22, 1992	49	F	55.0	20.5	12.33				
Jan 22, 1992	50	F	61.0	30.2	13.29	PN	117.8	2.82	Y
Jan 22, 1992	53	F	52.5	9.6	6.61				
Jan 22, 1992	54	F	53.0	12.4	8.30				
Jan 22, 1992	55	F	57.5	14.0	7.35	PN	113.7	5.33	N
Jan 22, 1992	57	F	57.5	18.6	9.79				
Jan 22, 1992	58	F	59.0	23.5	11.44	PN	126.6	5.32	Y
Jan 22, 1992	59	F	51.0	9.3	7.04				
Jan 22, 1992	60	F	52.0	7.9	5.62	PN	127.8	7.54	N
Jan 22, 1992	61	F	68.0	55.3	17.58	PN	113.1	2.69	Y
Jan 22, 1992	62	F	50.5	9.2	7.17	PN	125.2	9.07	Y
Jan 22, 1992	65	F	55.5	16.0	9.34				
Jan 22, 1992	66	F	55.0	11.9	7.13	PN	125.6	6.36	Y

APPENDIX 5. Reproductive data from wild-caught striped trumpeter, Latris lineata.

DATE	SAMPLE NUMBER	SEX	FORK LTH (cm)	GONAD WT.(g)	GONAD INDEX	MAX.DEV. STAGE	MEAN MAX. 10 OOCYTES	S.D. (+/-)	ATRETIC OOCYTES
Jan 22, 1992	68	F	55.0	9.9	5.95				
Jan 22, 1992	69	F	53.0	9.6	6.44				
Jan 22, 1992	71	F	62.5	27.4	11.21	PN	118.2	4.49	Y
Jan 22, 1992	76	F	56.5	20.3	11.23		108.2	3.12	
Jan 22, 1992	1	M	51.5	2.5	1.83				
Jan 22, 1992	2	M	52.5	1.7	1.15	DV			
Jan 22, 1992	3	M	56.5	3.5	1.95				
Jan 22, 1992	9	M	53.0	2.2	1.46				
Jan 22, 1992	10	M	69.0	15.3	4.66				
Jan 22, 1992	13	M	63.5	20.3	7.94	SR			
Jan 22, 1992	15	M	56.5	9.8	5.42				
Jan 22, 1992	17	M	52.0	2.9	2.05	SR			
Jan 22, 1992	20	M	56.0	4.8	2.74				
Jan 22, 1992	26	M	59.0	8.1	3.93	SR			
Jan 22, 1992	27	M	54.0	1.6	1.04				
Jan 22, 1992	28	M	63.0	9.2	3.66				
Jan 22, 1992	30	M	57.0	3.7	1.99				
Jan 22, 1992	31	M	54.5	4.1	2.53				
Jan 22, 1992	33	M	53.0	2.1	1.42				
Jan 22, 1992	34	M	56.5	4.7	2.61				
Jan 22, 1992	37	M	55.5	4.8	2.83				
Jan 22, 1992	38	M	60.0	3.4	1.56				
Jan 22, 1992	41	M	59.5	6.0	2.83				
Jan 22, 1992	48	M	61.5	9.1	3.90	SR			
Jan 22, 1992	51	M	58.0	4.0	2.05				
Jan 22, 1992	52	M	61.5	13.7	5.90	SR			
Jan 22, 1992	56	M	66.0	6.0	2.08				
Jan 22, 1992	63	M	51.5	3.9	2.88				
Jan 22, 1992	64	M	61.0	9.4	4.13	SR			
Jan 22, 1992	67	M	53.5	4.6	3.03	SR			
Jan 22, 1992	70	M	53.5	3.6	2.36				
Jan 22, 1992	72	M	51.0	6.1	4.58	SR			
Jan 22, 1992	73	M	54.5	2.0	1.26	DV			
Jan 22, 1992	74	M	50.0	3.0	2.40				
Jan 22, 1992	77	M	50.5	5.3	4.11	SR			
Feb 25, 1992	1	F	64.0	33.8	12.91	PN			Y
Feb 25, 1992	3	F	52.5	12.4	8.58	PN	118.3	2.31	N
Feb 25, 1992	4	F	58.0	30.2	15.49	PN	136.8	5.20	Y
Feb 25, 1992	6	F	62.5	33.2	13.60	PN	134.4	3.98	Y
Feb 25, 1992	11	F	54.0	17.2	10.93	PN	123.0	5.08	N
Feb 25, 1992	12	F	57.0	14.6	7.91	PN	128.4	7.58	N
Feb 25, 1992	14	F	85.0	122.1	19.88	PN	129.0	6.77	Y
Feb 25, 1992	18	F	80.5	93.8	17.98	PN	119.9	2.60	Y
Feb 25, 1992	19	F	56.0	24.5	13.95	PN	124.0	3.65	Y
Feb 25, 1992	20	F	55.0	17.0	10.23	PN	128.5	6.26	Y
Feb 25, 1992	21	F	55.0	12.1	7.30	PN	130.5	6.02	Y
Feb 25, 1992	23	F	66.0	29.3	10.19	PN	115.4	6.08	Y
Feb 25, 1992	24	F	52.0	16.2	11.49	PN	118.4	3.95	Y
Feb 25, 1992	25	F	54.0	12.0	7.59	PN	113.0	3.94	Y
Feb 25, 1992	29	F	61.0	32.5	14.31	PN	125.9	3.35	Y
Feb 25, 1992	30	F	56.0	17.7	10.05	PN	119.3	4.45	Y
Feb 25, 1992	31	F	53.0	21.0	14.11	PN	120.5	3.63	Y
Feb 25, 1992	35	F	50.0	10.9	8.71				
Feb 25, 1992	37	F	57.0	17.1	9.22	PN	126.2	6.80	Y
Feb 25, 1992	40	F	59.5	20.6	9.78	PN	129.3	2.45	Y
Feb 25, 1992	2	M	51.5	1.7	1.22	DV			
Feb 25, 1992	5	M	48.5	1.3	1.15	SR			
Feb 25, 1992	7	M	42.5	0.2	0.27	I			
Feb 25, 1992	8	M	61.0	10.0	4.41	SR			
Feb 25, 1992	9	M	53.5	2.1	1.37	DV			
Feb 25, 1992	10	M	55.5	3.5	2.04	DV			
Feb 25, 1992	13	M	55.0	6.2	3.71	SR			

APPENDIX 5. Reproductive data from wild-caught striped trumpeter, Latris lineata.

DATE	SAMPLE NUMBER	SEX	FORK LTH (cm)	GONAD WT.(g)	GONAD INDEX	MAX.DEV. STAGE	MEAN MAX. 10 OOCYTES	S.D. (+/-)	ATRETIC OOCYTES
Feb 25, 1992	15	M	55.0	6.5	3.92	M1			
Feb 25, 1992	16	M	58.0	6.4	3.30	SR			
Feb 25, 1992	17	M	54.5	7.2	4.47	SR			
Feb 25, 1992	22	M	56.0	3.8	2.15	SR			
Feb 25, 1992	26	M	64.0	8.9	3.40				
Feb 25, 1992	27	M	55.0	1.6	0.97	DV			
Feb 25, 1992	28	M	59.5	5.6	2.64	SR			
Feb 25, 1992	32	M	56.5	6.4	3.55	SR			
Feb 25, 1992	33	M	55.0	2.2	1.35	DV			
Feb 25, 1992	34	M	57.5	4.5	2.35	SR			
Feb 25, 1992	36	M	61.0	8.2	3.60				
Feb 25, 1992	38	M	59.0	6.9	3.38	SR			
Feb 25, 1992	39	M	54.5	3.0	1.83	SR			
May 22, 1992	1	F	66.5	49.5	16.83	PN	128.6	6.69	
May 22, 1992	4	F	52.5	12.7	8.78	CA	229.7	17.00	
May 22, 1992	7	F	57.0	23.1	12.47	CA	184.0	10.13	Y
May 22, 1992	2	M	66.0	9.7	3.37	M1			
May 22, 1992	3	M	52.5	2.2	1.52	M1			
May 22, 1992	5	M	53.5	1.6	1.04	M1			
May 22, 1992	6	M	56.5	10.0	5.54	M1			
May 22, 1992	8	M	47.5	0.7	0.65	I			
May 22, 1992	9	M	53.5	3.3	2.16	M1			
May 22, 1992	10	M	57.0	3.3	1.78				
May 22, 1992	11	M	63.0	5.0	2.00	SR			

APPENDIX 6. Projected age and fork length data for wild-caught striped trumpeter, Latris lineata.

SAMPLING DATE	SAMPLE NUMBER	SEX	FORK LTH (cm)	Log10 F.L.	AGE ADJ. BACK	AGE ADJ. FORWARD	AGE AT SAMPLING	AGE PREV. SPAWN	F.L. PREV. SPAWN	AGE NEXT SPAWN	F.L. NEXT SPAWN
May 25, 1990	2	F	59.5	1.7745	0.650	0.35	10.57	9.92	58.6	10.92	60.65
May 25, 1990	6	F	80.0	1.9031	0.650	0.35	22.58	21.93	75.3	22.93	76.26
May 25, 1990	8	F	61.0	1.7853	0.650	0.35	11.32	10.67	60.2	11.67	62.05
May 25, 1990	10	F	49.5	1.6946	0.650	0.35	6.51	5.86	47.5	6.86	50.85
May 25, 1990	11	F	59.5	1.7745	0.650	0.35	10.57	9.92	58.6	10.92	60.65
May 25, 1990	14	F	71.0	1.8513	0.650	0.35	16.93	16.28	69.0	17.28	70.30
May 25, 1990	16	F	61.0	1.7853	0.650	0.35	11.32	10.67	60.2	11.67	62.05
May 25, 1990	17	F	49.0	1.6902	0.650	0.35	6.36	5.71	47.0	6.71	50.39
May 25, 1990	18	F	49.0	1.6902	0.650	0.35	6.36	5.71	47.0	6.71	50.39
May 25, 1990	20	F	53.5	1.7284	0.650	0.35	7.90	7.25	52.0	8.25	54.76
May 25, 1990	1	M	66.0	1.8195	0.650	0.35	14.01	13.36	64.9	14.36	66.41
May 25, 1990	3	M	60.0	1.7782	0.650	0.35	10.82	10.17	59.1	11.17	61.12
May 25, 1990	4	M	69.0	1.8388	0.650	0.35	15.74	15.09	67.4	16.09	68.80
May 25, 1990	5	M	55.5	1.7443	0.650	0.35	8.73	8.08	54.3	9.08	56.76
May 25, 1990	7	M	66.0	1.8195	0.650	0.35	14.01	13.36	64.9	14.36	66.41
May 25, 1990	9	M	58.5	1.7672	0.650	0.35	10.09	9.44	57.6	10.44	59.70
May 25, 1990	12	M	60.0	1.7782	0.650	0.35	10.82	10.17	59.1	11.17	61.12
May 25, 1990	13	M	63.5	1.8028	0.650	0.35	12.63	11.98	62.6	12.98	64.29
May 25, 1990	15	M	53.0	1.7243	0.650	0.35	7.71	7.06	51.5	8.06	54.26
May 25, 1990	19	M	54.0	1.7324	0.650	0.35	8.10	7.45	52.6	8.45	55.26
Jul 12, 1990	22	F	65.0	1.8129	0.780	0.22	13.45	12.67	63.8	13.67	65.37
Jul 12, 1990	23	F	82.0	1.9138	0.780	0.22	23.89	23.11	76.4	24.11	77.31
Jul 12, 1990	24	F	62.0	1.7924	0.780	0.22	11.84	11.06	60.9	12.06	62.73
Jul 12, 1990	26	F	61.0	1.7853	0.780	0.22	11.32	10.54	59.9	11.54	61.81
Jul 12, 1990	27	F	59.0	1.7709	0.780	0.22	10.33	9.55	57.8	10.55	59.92
Jul 12, 1990	30	F	62.0	1.7924	0.780	0.22	11.84	11.06	60.9	12.06	62.73
Jul 12, 1990	32	F	65.0	1.8129	0.780	0.22	13.45	12.67	63.8	13.67	65.37
Jul 12, 1990	33	F	63.0	1.7993	0.780	0.22	12.36	11.58	61.9	12.58	63.63
Jul 12, 1990	37	F	66.5	1.8228	0.780	0.22	14.29	13.51	65.1	14.51	66.63
Jul 12, 1990	42	F	55.0	1.7404	0.780	0.22	8.51	7.73	53.4	8.73	55.95
Jul 12, 1990	43	F	60.0	1.7782	0.780	0.22	10.82	10.04	58.9	11.04	60.87
Jul 12, 1990	44	F	60.0	1.7782	0.780	0.22	10.82	10.04	58.9	11.04	60.87
Jul 12, 1990	21	M	64.0	1.8062	0.780	0.22	12.90	12.12	62.8	13.12	64.51
Jul 12, 1990	25	M	51.5	1.7118	0.780	0.22	7.16	6.38	49.3	7.38	52.41
Jul 12, 1990	28	M	51.0	1.7076	0.780	0.22	6.99	6.21	48.8	7.21	51.91
Jul 12, 1990	29	M	75.0	1.8751	0.780	0.22	19.39	18.61	71.9	19.61	72.96
Jul 12, 1990	31	M	58.5	1.7672	0.780	0.22	10.09	9.31	57.3	10.31	59.43
Jul 12, 1990	34	M	54.5	1.7364	0.780	0.22	8.31	7.53	52.8	8.53	55.44
Jul 12, 1990	35	M	66.5	1.8228	0.780	0.22	14.29	13.51	65.1	14.51	66.63
Jul 12, 1990	36	M	58.0	1.7634	0.780	0.22	9.85	9.07	56.7	10.07	58.94
Jul 12, 1990	38	M	62.5	1.7959	0.780	0.22	12.10	11.32	61.4	12.32	63.18
Jul 12, 1990	39	M	56.0	1.7482	0.780	0.22	8.94	8.16	54.5	9.16	56.96
Jul 12, 1990	40	M	55.5	1.7443	0.780	0.22	8.73	7.95	54.0	8.95	56.45
Jul 12, 1990	41	M	54.5	1.7364	0.780	0.22	8.31	7.53	52.8	8.53	55.44
Jul 12, 1990	45	M	54.5	1.7364	0.780	0.22	8.31	7.53	52.8	8.53	55.44
Sep 20, 1990	1	F	58.5	1.7672	0.970	0.03	10.09	9.12	56.9	10.12	59.04
Sep 20, 1990	2	F	62.0	1.7924	0.970	0.03	11.84	10.87	60.5	11.87	62.40
Sep 20, 1990	4	F	58.5	1.7672	0.970	0.03	10.09	9.12	56.9	10.12	59.04
Sep 20, 1990	7	F	54.0	1.7324	0.970	0.03	8.10	7.13	51.7	8.13	54.45
Sep 20, 1990	17	F	58.5	1.7672	0.970	0.03	10.09	9.12	56.9	10.12	59.04
Sep 20, 1990	18	F	56.5	1.7520	0.970	0.03	9.16	8.19	54.6	9.19	57.03
Sep 20, 1990	20	F	61.0	1.7853	0.970	0.03	11.32	10.35	59.5	11.35	61.46
Sep 20, 1990	22	F	56.0	1.7482	0.970	0.03	8.94	7.97	54.0	8.97	56.52
Sep 20, 1990	24	F	54.5	1.7364	0.970	0.03	8.31	7.34	52.3	8.34	54.97
Sep 20, 1990	25	F	55.5	1.7443	0.970	0.03	8.73	7.76	53.5	8.76	56.00
Sep 20, 1990	26	F	56.5	1.7520	0.970	0.03	9.16	8.19	54.6	9.19	57.03
Sep 20, 1990	27	F	55.5	1.7443	0.970	0.03	8.73	7.76	53.5	8.76	56.00
Sep 20, 1990	29	F	54.0	1.7324	0.970	0.03	8.10	7.13	51.7	8.13	54.45
Sep 20, 1990	30	F	56.0	1.7482	0.970	0.03	8.94	7.97	54.0	8.97	56.52
Sep 20, 1990	35	F	57.0	1.7559	0.970	0.03	9.39	8.42	55.2	9.42	57.54
Sep 20, 1990	36	F	70.5	1.8482	0.970	0.03	16.63	15.66	68.2	16.66	69.53
Sep 20, 1990	38	F	54.0	1.7324	0.970	0.03	8.10	7.13	51.7	8.13	54.45
Sep 20, 1990	40	F	56.0	1.7482	0.970	0.03	8.94	7.97	54.0	8.97	56.52
Sep 20, 1990	21	M	56.0	1.7482	0.970	0.03	8.94	7.97	54.0	8.97	56.52
Sep 20, 1990	23	M	52.5	1.7202	0.970	0.03	7.52	6.55	49.9	7.55	52.89
Sep 20, 1990	31	M	61.5	1.7889	0.970	0.03	11.58	10.61	60.0	11.61	61.93
Sep 20, 1990	32	M	56.0	1.7482	0.970	0.03	8.94	7.97	54.0	8.97	56.52
Sep 20, 1990	33	M	58.0	1.7634	0.970	0.03	9.85	8.88	56.3	9.88	58.54
Sep 20, 1990	37	M	59.5	1.7745	0.970	0.03	10.57	9.60	57.9	10.60	60.02
Sep 20, 1990	39	M	51.0	1.7076	0.970	0.03	6.99	6.02	48.1	7.02	51.35
Sep 29, 1990	2	F	63.0	1.7993	0.997	0.00	12.36	11.37	61.5	12.37	63.27
Sep 29, 1990	6	F	52.5	1.7202	0.997	0.00	7.52	6.53	49.8	7.53	52.81
Sep 29, 1990	7	F	68.0	1.8325	0.997	0.00	15.15	14.15	66.1	15.15	67.54

APPENDIX 6. Projected age and fork length data for wild-caught striped trumpeter, Latris lineata.

SAMPLING DATE	SAMPLE NUMBER	SEX	FORK LTH (cm)	Log10 F.L.	AGE ADJ. BACK	AGE ADJ. FORWARD	AGE AT SAMPLING	AGE PREV. SPAWN	F.L. PREV. SPAWN	AGE NEXT SPAWN	F.L. NEXT SPAWN
Sep 29, 1990	8	F	84.0	1.9243	0.997	0.00	25.21	24.21	77.4	25.21	78.25
Sep 29, 1990	9	F	70.0	1.8451	0.997	0.00	16.33	15.33	67.8	16.33	69.12
Sep 29, 1990	10	F	50.0	1.6990	0.997	0.00	6.66	5.66	46.8	6.66	50.26
Sep 29, 1990	11	F	60.5	1.7818	0.997	0.00	11.07	10.07	58.9	11.07	60.94
Sep 29, 1990	12	F	51.0	1.7076	0.997	0.00	6.99	5.99	48.0	6.99	51.26
Sep 29, 1990	17	F	66.0	1.8195	0.997	0.00	14.01	13.01	64.3	14.01	65.89
Sep 29, 1990	20	F	52.0	1.7160	0.997	0.00	7.34	6.34	49.2	7.34	52.29
Sep 29, 1990	22	F	65.0	1.8129	0.997	0.00	13.45	12.45	63.4	13.45	65.04
Sep 29, 1990	29	F	56.0	1.7482	0.997	0.00	8.94	7.95	54.0	8.95	56.45
Sep 29, 1990	30	F	72.5	1.8603	0.997	0.00	17.84	16.84	69.8	17.84	70.98
Sep 29, 1990	31	F	53.0	1.7243	0.997	0.00	7.71	6.71	50.4	7.71	53.33
Sep 29, 1990	32	F	78.5	1.8949	0.997	0.00	21.61	20.61	74.0	21.61	75.01
Sep 29, 1990	39	F	65.5	1.8162	0.997	0.00	13.73	12.73	63.9	13.73	65.47
Sep 29, 1990	42	F	52.0	1.7160	0.997	0.00	7.34	6.34	49.2	7.34	52.29
Sep 29, 1990	1	M	62.0	1.7924	0.997	0.00	11.84	10.84	60.5	11.84	62.35
Sep 29, 1990	3	M	59.0	1.7709	0.997	0.00	10.33	9.33	57.3	10.33	59.48
Sep 29, 1990	4	M	58.0	1.7634	0.997	0.00	9.85	8.85	56.2	9.85	58.49
Sep 29, 1990	5	M	61.5	1.7889	0.997	0.00	11.58	10.58	60.0	11.58	61.88
Sep 29, 1990	13	M	52.0	1.7160	0.997	0.00	7.34	6.34	49.2	7.34	52.29
Sep 29, 1990	14	M	58.5	1.7672	0.997	0.00	10.09	9.09	56.8	10.09	58.98
Sep 29, 1990	15	M	58.5	1.7672	0.997	0.00	10.09	9.09	56.8	10.09	58.98
Sep 29, 1990	16	M	54.5	1.7364	0.997	0.00	8.31	7.31	52.2	8.31	54.90
Sep 29, 1990	18	M	58.0	1.7634	0.997	0.00	9.85	8.85	56.2	9.85	58.49
Sep 29, 1990	19	M	52.0	1.7160	0.997	0.00	7.34	6.34	49.2	7.34	52.29
Sep 29, 1990	21	M	53.0	1.7243	0.997	0.00	7.71	6.71	50.4	7.71	53.33
Sep 29, 1990	23	M	54.0	1.7324	0.997	0.00	8.10	7.11	51.6	8.11	54.38
Sep 29, 1990	24	M	56.5	1.7520	0.997	0.00	9.16	8.17	54.5	9.17	56.97
Sep 29, 1990	25	M	54.0	1.7324	0.997	0.00	8.10	7.11	51.6	8.11	54.38
Sep 29, 1990	26	M	55.0	1.7404	0.997	0.00	8.51	7.52	52.8	8.52	55.42
Sep 29, 1990	27	M	57.0	1.7559	0.997	0.00	9.39	8.39	55.1	9.39	57.48
Sep 29, 1990	28	M	63.5	1.8028	0.997	0.00	12.63	11.63	62.0	12.63	63.72
Sep 29, 1990	33	M	56.5	1.7520	0.997	0.00	9.16	8.17	54.5	9.17	56.97
Sep 29, 1990	34	M	50.0	1.6990	0.997	0.00	6.66	5.66	46.8	6.66	50.26
Sep 29, 1990	35	M	51.5	1.7118	0.997	0.00	7.16	6.16	48.6	7.16	51.78
Sep 29, 1990	36	M	50.5	1.7033	0.997	0.00	6.82	5.82	47.4	6.82	50.76
Sep 29, 1990	37	M	67.5	1.8293	0.997	0.00	14.86	13.87	65.7	14.87	67.14
Sep 29, 1990	38	M	50.0	1.6990	0.997	0.00	6.66	5.66	46.8	6.66	50.26
Sep 29, 1990	40	M	62.5	1.7959	0.997	0.00	12.10	11.10	61.0	12.10	62.81
Sep 29, 1990	41	M	59.0	1.7709	0.997	0.00	10.33	9.33	57.3	10.33	59.48
Sep 29, 1990	43	M	58.0	1.7634	0.997	0.00	9.85	8.85	56.2	9.85	58.49
Sep 29, 1990	44	M	56.0	1.7482	0.997	0.00	8.94	7.95	54.0	8.95	56.45
Sep 29, 1990	45	M	58.0	1.7634	0.997	0.00	9.85	8.85	56.2	9.85	58.49
Mar 14, 1991	2	F	77.0	1.8865	0.530	0.47	20.65	20.12	73.5	21.12	74.53
Mar 14, 1991	3	F	72.0	1.8573	0.530	0.47	17.53	17.00	70.0	18.00	71.17
Mar 14, 1991	7	F	55.5	1.7443	0.530	0.47	8.73	8.20	54.6	9.20	57.03
Mar 14, 1991	9	F	55.0	1.7404	0.530	0.47	8.51	7.98	54.1	8.98	56.54
Mar 14, 1991	11	F	49.5	1.6946	0.530	0.47	6.51	5.98	48.0	6.98	51.22
Mar 14, 1991	12	F	55.0	1.7404	0.530	0.47	8.51	7.98	54.1	8.98	56.54
Mar 14, 1991	18	F	68.5	1.8357	0.530	0.47	15.44	14.91	67.2	15.91	68.57
Mar 14, 1991	23	F	52.5	1.7202	0.530	0.47	7.52	6.99	51.3	7.99	54.08
Mar 14, 1991	26	F	49.0	1.6902	0.530	0.47	6.36	5.83	47.4	6.83	50.77
Mar 14, 1991	30	F	52.0	1.7160	0.530	0.47	7.34	6.81	50.7	7.81	53.59
Mar 14, 1991	31	F	52.5	1.7202	0.530	0.47	7.52	6.99	51.3	7.99	54.08
Mar 14, 1991	32	F	56.0	1.7482	0.530	0.47	8.94	8.41	55.2	9.41	57.52
Mar 14, 1991	34	F	48.5	1.6857	0.530	0.47	6.22	5.69	46.9	6.69	50.32
Mar 14, 1991	36	F	84.0	1.9243	0.530	0.47	25.21	24.68	77.8	25.68	78.64
Mar 14, 1991	37	F	59.5	1.7745	0.530	0.47	10.57	10.04	58.9	11.04	60.88
Mar 14, 1991	39	F	74.0	1.8692	0.530	0.47	18.76	18.23	71.4	19.23	72.56
Mar 14, 1991	40	F	65.0	1.8129	0.530	0.47	13.45	12.92	64.2	13.92	65.76
Mar 14, 1991	41	F	70.5	1.8482	0.530	0.47	16.63	16.10	68.8	17.10	70.08
Mar 14, 1991	42	F	56.0	1.7482	0.530	0.47	8.94	8.41	55.2	9.41	57.52
Mar 14, 1991	45	F	83.0	1.9191	0.530	0.47	24.54	24.01	77.2	25.01	78.09
Mar 14, 1991	46	F	70.5	1.8482	0.530	0.47	16.63	16.10	68.8	17.10	70.08
Mar 14, 1991	48	F	63.0	1.7993	0.530	0.47	12.36	11.83	62.3	12.83	64.05
Mar 14, 1991	49	F	54.0	1.7324	0.530	0.47	8.10	7.57	52.9	8.57	55.56
Mar 14, 1991	53	F	61.5	1.7889	0.530	0.47	11.58	11.05	60.9	12.05	62.71
Mar 14, 1991	54	F	66.0	1.8195	0.530	0.47	14.01	13.48	65.1	14.48	66.58
Mar 14, 1991	1	M	64.0	1.8062	0.530	0.47	12.90	12.37	63.3	13.37	64.91
Mar 14, 1991	4	M	64.0	1.8062	0.530	0.47	12.90	12.37	63.3	13.37	64.91
Mar 14, 1991	6	M	50.0	1.6990	0.530	0.47	6.66	6.13	48.5	7.13	51.68
Mar 14, 1991	8	M	65.5	1.8162	0.530	0.47	13.73	13.20	64.6	14.20	66.17
Mar 14, 1991	10	M	64.0	1.8062	0.530	0.47	12.90	12.37	63.3	13.37	64.91
Mar 14, 1991	14	M	52.0	1.7160	0.530	0.47	7.34	6.81	50.7	7.81	53.59

APPENDIX 6. Projected age and fork length data for wild-caught striped trumpeter, Latris lineata.

SAMPLING DATE	SAMPLE NUMBER	SEX	FORK LTH (cm)	Log10 F.L.	AGE ADJ. BACK	AGE ADJ. FORWARD	AGE AT SAMPLING	AGE PREV. SPAWN	F.L. PREV. SPAWN	AGE NEXT SPAWN	F.L. NEXT SPAWN
Mar 14, 1991	15	M	64.0	1.8062	0.530	0.47	12.90	12.37	63.3	13.37	64.91
Mar 14, 1991	16	M	55.0	1.7404	0.530	0.47	8.51	7.98	54.1	8.98	56.54
Mar 14, 1991	17	M	71.0	1.8513	0.530	0.47	16.93	16.40	69.2	17.40	70.45
Mar 14, 1991	19	M	53.0	1.7243	0.530	0.47	7.71	7.18	51.8	8.18	54.57
Mar 14, 1991	20	M	61.0	1.7853	0.530	0.47	11.32	10.79	60.4	11.79	62.26
Mar 14, 1991	21	M	53.0	1.7243	0.530	0.47	7.71	7.18	51.8	8.18	54.57
Mar 14, 1991	22	M	52.5	1.7202	0.530	0.47	7.52	6.99	51.3	7.99	54.08
Mar 14, 1991	24	M	56.5	1.7520	0.530	0.47	9.16	8.63	55.7	9.63	58.01
Mar 14, 1991	25	M	58.0	1.7634	0.530	0.47	9.85	9.32	57.3	10.32	59.46
Mar 14, 1991	27	M	55.0	1.7404	0.530	0.47	8.51	7.98	54.1	8.98	56.54
Mar 14, 1991	28	M	52.5	1.7202	0.530	0.47	7.52	6.99	51.3	7.99	54.08
Mar 14, 1991	29	M	54.5	1.7364	0.530	0.47	8.31	7.78	53.5	8.78	56.05
Mar 14, 1991	33	M	55.0	1.7404	0.530	0.47	8.51	7.98	54.1	8.98	56.54
Mar 14, 1991	35	M	69.5	1.8420	0.530	0.47	16.03	15.50	68.0	16.50	69.33
Mar 14, 1991	38	M	71.0	1.8513	0.530	0.47	16.93	16.40	69.2	17.40	70.45
Mar 14, 1991	47	M	65.0	1.8129	0.530	0.47	13.45	12.92	64.2	13.92	65.76
Mar 14, 1991	50	M	53.0	1.7243	0.530	0.47	7.71	7.18	51.8	8.18	54.57
Mar 14, 1991	51	M	49.5	1.6946	0.530	0.47	6.51	5.98	48.0	6.98	51.22
Mar 14, 1991	52	M	72.0	1.8573	0.530	0.47	17.53	17.00	70.0	18.00	71.17
Jun 14, 1991	2	F	56.5	1.7520	0.700	0.30	9.16	8.46	55.3	9.46	57.64
Jun 14, 1991	3	F	50.5	1.7033	0.700	0.30	6.82	6.12	48.5	7.12	51.65
Jun 14, 1991	5	F	54.5	1.7364	0.700	0.30	8.31	7.61	53.0	8.61	55.64
Jun 14, 1991	6	F	50.0	1.6990	0.700	0.30	6.66	5.96	47.9	6.96	51.17
Jun 14, 1991	8	F	50.0	1.6990	0.700	0.30	6.66	5.96	47.9	6.96	51.17
Jun 14, 1991	9	F	76.0	1.8808	0.700	0.30	20.02	19.32	72.6	20.32	73.71
Jun 14, 1991	10	F	65.5	1.8162	0.700	0.30	13.73	13.03	64.4	14.03	65.92
Jun 14, 1991	13	F	52.5	1.7202	0.700	0.30	7.52	6.82	50.7	7.82	53.63
Jun 14, 1991	16	F	47.5	1.6767	0.700	0.30	5.95	5.25	45.2	6.25	48.92
Jun 14, 1991	17	F	53.5	1.7284	0.700	0.30	7.90	7.20	51.9	8.20	54.63
Jun 14, 1991	18	F	53.0	1.7243	0.700	0.30	7.71	7.01	51.3	8.01	54.13
Jun 14, 1991	19	F	52.0	1.7160	0.700	0.30	7.34	6.64	50.2	7.64	53.13
Jun 14, 1991	1	M	65.0	1.8129	0.700	0.30	13.45	12.75	63.9	13.75	65.50
Jun 14, 1991	4	M	50.0	1.6990	0.700	0.30	6.66	5.96	47.9	6.96	51.17
Jun 14, 1991	7	M	60.0	1.7782	0.700	0.30	10.82	10.12	59.0	11.12	61.02
Jun 14, 1991	11	M	57.0	1.7559	0.700	0.30	9.39	8.69	55.8	9.69	58.13
Jun 14, 1991	12	M	53.5	1.7284	0.700	0.30	7.90	7.20	51.9	8.20	54.63
Jun 14, 1991	14	M	46.0	1.6628	0.700	0.30	5.61	4.91	43.8	5.91	47.74
Jun 14, 1991	15	M	58.5	1.7672	0.700	0.30	10.09	9.39	57.5	10.39	59.59
Jun 14, 1991	20	M	53.0	1.7243	0.700	0.30	7.71	7.01	51.3	8.01	54.13
Jun 14, 1991	21	M	61.0	1.7853	0.700	0.30	11.32	10.62	60.1	11.62	61.96
Oct 4, 1991	37	F	62.0	1.7924	1.010	-0.01	11.84	10.83	60.5	11.83	62.33
Oct 4, 1991	38	F	65.5	1.8162	1.010	-0.01	13.73	12.72	63.9	13.72	65.45
Oct 4, 1991	40	F	78.5	1.8949	1.010	-0.01	21.61	20.60	74.0	21.60	75.00
Oct 4, 1991	46	F	56.0	1.7482	1.010	-0.01	8.94	7.93	53.9	8.93	56.42
Oct 4, 1991	47	F	54.5	1.7364	1.010	-0.01	8.31	7.30	52.2	8.30	54.87
Oct 4, 1991	49	F	51.5	1.7118	1.010	-0.01	7.16	6.15	48.6	7.15	51.74
Oct 4, 1991	33	F	54.5	1.7364	1.010	-0.01	8.31	7.30	52.2	8.30	54.87
Oct 4, 1991	35	F	64.5	1.8096	1.010	-0.01	13.17	12.16	62.9	13.16	64.58
Oct 4, 1991	36	F	83.0	1.9191	1.010	-0.01	24.54	23.53	76.8	24.53	77.68
Oct 4, 1991	39	F	54.0	1.7324	1.010	-0.01	8.10	7.09	51.6	8.09	54.34
Oct 4, 1991	43	F	67.5	1.8293	1.010	-0.01	14.86	13.85	65.7	14.85	67.12
Oct 4, 1991	52	F	54.0	1.7324	1.010	-0.01	8.10	7.09	51.6	8.09	54.34
Oct 4, 1991	41	M	58.5	1.7672	1.010	-0.01	10.09	9.08	56.8	10.08	58.96
Oct 4, 1991	48	M	60.0	1.7782	1.010	-0.01	10.82	9.81	58.4	10.81	60.43
Oct 4, 1991	50	M	62.0	1.7924	1.010	-0.01	11.84	10.83	60.5	11.83	62.33
Oct 4, 1991	51	M	58.5	1.7672	1.010	-0.01	10.09	9.08	56.8	10.08	58.96
Oct 4, 1991	53	M	56.5	1.7520	1.010	-0.01	9.16	8.15	54.5	9.15	56.94
Oct 4, 1991	34	M	68.0	1.8325	1.010	-0.01	15.15	14.14	66.1	15.14	67.53
Oct 4, 1991	42	M	66.5	1.8228	1.010	-0.01	14.29	13.28	64.8	14.28	66.29
Oct 4, 1991	45	M	59.5	1.7745	1.010	-0.01	10.57	9.56	57.8	10.56	59.94
Jan 22, 1992	4	F	60.0	1.7782	0.310	0.69	10.82	10.51	59.8	11.51	61.75
Jan 22, 1992	5	F	58.5	1.7672	0.310	0.69	10.09	9.78	58.3	10.78	60.37
Jan 22, 1992	6	F	53.5	1.7284	0.310	0.69	7.90	7.59	53.0	8.59	55.61
Jan 22, 1992	7	F	56.5	1.7520	0.310	0.69	9.16	8.85	56.2	9.85	58.49
Jan 22, 1992	8	F	57.0	1.7559	0.310	0.69	9.39	9.08	56.8	10.08	58.96
Jan 22, 1992	11	F	51.0	1.7076	0.310	0.69	6.99	6.68	50.3	7.68	53.24
Jan 22, 1992	12	F	52.0	1.7160	0.310	0.69	7.34	7.03	51.4	8.03	54.18
Jan 22, 1992	14	F	43.5	1.6385	0.310	0.69	5.21	4.90	43.8	5.90	47.69
Jan 22, 1992	16	F	54.0	1.7324	0.310	0.69	8.10	7.79	53.5	8.79	56.09
Jan 22, 1992	18	F	55.5	1.7443	0.310	0.69	8.73	8.42	55.2	9.42	57.53
Jan 22, 1992	19	F	58.0	1.7634	0.310	0.69	9.85	9.54	57.8	10.54	59.90
Jan 22, 1992	21	F	52.5	1.7202	0.310	0.69	7.52	7.21	51.9	8.21	54.65
Jan 22, 1992	22	F	66.0	1.8195	0.310	0.69	14.01	13.70	65.4	14.70	66.90

APPENDIX 6. Projected age and fork length data for wild-caught striped trumpeter, Latris lineata.

SAMPLING DATE	SAMPLE NUMBER	SEX	FORK LTH (cm)	Log10 F.L.	AGE ADJ. BACK	AGE ADJ. FORWARD	AGE AT SAMPLING	AGE PREV. SPAWN	F.L. PREV. SPAWN	AGE NEXT SPAWN	F.L. NEXT SPAWN
Jan 22, 1992	23	F	56.0	1.7482	0.310	0.69	8.94	8.63	55.7	9.63	58.01
Jan 22, 1992	24	F	66.0	1.8195	0.310	0.69	14.01	13.70	65.4	14.70	66.90
Jan 22, 1992	25	F	49.0	1.6902	0.310	0.69	6.36	6.05	48.2	7.05	51.43
Jan 22, 1992	29	F	61.0	1.7853	0.310	0.69	11.32	11.01	60.8	12.01	62.65
Jan 22, 1992	32	F	53.5	1.7284	0.310	0.69	7.90	7.59	53.0	8.59	55.61
Jan 22, 1992	35	F	52.0	1.7160	0.310	0.69	7.34	7.03	51.4	8.03	54.18
Jan 22, 1992	36	F	54.0	1.7324	0.310	0.69	8.10	7.79	53.5	8.79	56.09
Jan 22, 1992	39	F	58.0	1.7634	0.310	0.69	9.85	9.54	57.8	10.54	59.90
Jan 22, 1992	40	F	55.0	1.7404	0.310	0.69	8.51	8.20	54.6	9.20	57.05
Jan 22, 1992	42	F	55.0	1.7404	0.310	0.69	8.51	8.20	54.6	9.20	57.05
Jan 22, 1992	43	F	58.0	1.7634	0.310	0.69	9.85	9.54	57.8	10.54	59.90
Jan 22, 1992	45	F	52.0	1.7160	0.310	0.69	7.34	7.03	51.4	8.03	54.18
Jan 22, 1992	46	F	69.0	1.8388	0.310	0.69	15.74	15.43	67.9	16.43	69.24
Jan 22, 1992	47	F	64.0	1.8062	0.310	0.69	12.90	12.59	63.6	13.59	65.25
Jan 22, 1992	49	F	55.0	1.7404	0.310	0.69	8.51	8.20	54.6	9.20	57.05
Jan 22, 1992	50	F	61.0	1.7853	0.310	0.69	11.32	11.01	60.8	12.01	62.65
Jan 22, 1992	53	F	52.5	1.7202	0.310	0.69	7.52	7.21	51.9	8.21	54.65
Jan 22, 1992	54	F	53.0	1.7243	0.310	0.69	7.71	7.40	52.5	8.40	55.13
Jan 22, 1992	55	F	57.5	1.7597	0.310	0.69	9.62	9.31	57.3	10.31	59.43
Jan 22, 1992	57	F	57.5	1.7597	0.310	0.69	9.62	9.31	57.3	10.31	59.43
Jan 22, 1992	58	F	59.0	1.7709	0.310	0.69	10.33	10.02	58.8	11.02	60.83
Jan 22, 1992	59	F	51.0	1.7076	0.310	0.69	6.99	6.68	50.3	7.68	53.24
Jan 22, 1992	60	F	52.0	1.7160	0.310	0.69	7.34	7.03	51.4	8.03	54.18
Jan 22, 1992	61	F	68.0	1.8325	0.310	0.69	15.15	14.84	67.1	15.84	68.48
Jan 22, 1992	62	F	50.5	1.7033	0.310	0.69	6.82	6.51	49.8	7.51	52.78
Jan 22, 1992	65	F	55.5	1.7443	0.310	0.69	8.73	8.42	55.2	9.42	57.53
Jan 22, 1992	66	F	55.0	1.7404	0.310	0.69	8.51	8.20	54.6	9.20	57.05
Jan 22, 1992	68	F	55.0	1.7404	0.310	0.69	8.51	8.20	54.6	9.20	57.05
Jan 22, 1992	69	F	53.0	1.7243	0.310	0.69	7.71	7.40	52.5	8.40	55.13
Jan 22, 1992	71	F	62.5	1.7959	0.310	0.69	12.10	11.79	62.3	12.79	63.97
Jan 22, 1992	76	F	56.5	1.7520	0.310	0.69	9.16	8.85	56.2	9.85	58.49
Jan 22, 1992	1	M	51.5	1.7118	0.310	0.69	7.16	6.85	50.8	7.85	53.70
Jan 22, 1992	2	M	52.5	1.7202	0.310	0.69	7.52	7.21	51.9	8.21	54.65
Jan 22, 1992	3	M	56.5	1.7520	0.310	0.69	9.16	8.85	56.2	9.85	58.49
Jan 22, 1992	9	M	53.0	1.7243	0.310	0.69	7.71	7.40	52.5	8.40	55.13
Jan 22, 1992	10	M	69.0	1.8388	0.310	0.69	15.74	15.43	67.9	16.43	69.24
Jan 22, 1992	13	M	63.5	1.8028	0.310	0.69	12.63	12.32	63.2	13.32	64.83
Jan 22, 1992	15	M	56.5	1.7520	0.310	0.69	9.16	8.85	56.2	9.85	58.49
Jan 22, 1992	17	M	52.0	1.7160	0.310	0.69	7.34	7.03	51.4	8.03	54.18
Jan 22, 1992	20	M	56.0	1.7482	0.310	0.69	8.94	8.63	55.7	9.63	58.01
Jan 22, 1992	26	M	59.0	1.7709	0.310	0.69	10.33	10.02	58.8	11.02	60.83
Jan 22, 1992	27	M	54.0	1.7324	0.310	0.69	8.10	7.79	53.5	8.79	56.09
Jan 22, 1992	28	M	63.0	1.7993	0.310	0.69	12.36	12.05	62.7	13.05	64.40
Jan 22, 1992	30	M	57.0	1.7559	0.310	0.69	9.39	9.08	56.8	10.08	58.96
Jan 22, 1992	31	M	54.5	1.7364	0.310	0.69	8.31	8.00	54.1	9.00	56.57
Jan 22, 1992	33	M	53.0	1.7243	0.310	0.69	7.71	7.40	52.5	8.40	55.13
Jan 22, 1992	34	M	56.5	1.7520	0.310	0.69	9.16	8.85	56.2	9.85	58.49
Jan 22, 1992	37	M	55.5	1.7443	0.310	0.69	8.73	8.42	55.2	9.42	57.53
Jan 22, 1992	38	M	60.0	1.7782	0.310	0.69	10.82	10.51	59.8	11.51	61.75
Jan 22, 1992	41	M	59.5	1.7745	0.310	0.69	10.57	10.26	59.3	11.26	61.29
Jan 22, 1992	48	M	61.5	1.7889	0.310	0.69	11.58	11.27	61.3	12.27	63.10
Jan 22, 1992	51	M	58.0	1.7634	0.310	0.69	9.85	9.54	57.8	10.54	59.90
Jan 22, 1992	52	M	61.5	1.7889	0.310	0.69	11.58	11.27	61.3	12.27	63.10
Jan 22, 1992	56	M	66.0	1.8195	0.310	0.69	14.01	13.70	65.4	14.70	66.90
Jan 22, 1992	63	M	51.5	1.7118	0.310	0.69	7.16	6.85	50.8	7.85	53.70
Jan 22, 1992	64	M	61.0	1.7853	0.310	0.69	11.32	11.01	60.8	12.01	62.65
Jan 22, 1992	67	M	53.5	1.7284	0.310	0.69	7.90	7.59	53.0	8.59	55.61
Jan 22, 1992	70	M	53.5	1.7284	0.310	0.69	7.90	7.59	53.0	8.59	55.61
Jan 22, 1992	72	M	51.0	1.7076	0.310	0.69	6.99	6.68	50.3	7.68	53.24
Jan 22, 1992	73	M	54.5	1.7364	0.310	0.69	8.31	8.00	54.1	9.00	56.57
Jan 22, 1992	74	M	50.0	1.6990	0.310	0.69	6.66	6.35	49.2	7.35	52.32
Jan 22, 1992	77	M	50.5	1.7033	0.310	0.69	6.82	6.51	49.8	7.51	52.78
Feb 25, 1992	1	F	64.0	1.8062	0.410	0.59	12.90	12.49	63.5	13.49	65.10
Feb 25, 1992	3	F	52.5	1.7202	0.410	0.59	7.52	7.11	51.6	8.11	54.39
Feb 25, 1992	4	F	58.0	1.7634	0.410	0.59	9.85	9.44	57.6	10.44	59.70
Feb 25, 1992	6	F	62.5	1.7959	0.410	0.59	12.10	11.69	62.1	12.69	63.81
Feb 25, 1992	11	F	54.0	1.7324	0.410	0.59	8.10	7.69	53.3	8.69	55.85
Feb 25, 1992	12	F	57.0	1.7559	0.410	0.59	9.39	8.98	56.5	9.98	58.75
Feb 25, 1992	14	F	85.0	1.9294	0.410	0.59	25.87	25.46	78.5	26.46	79.27
Feb 25, 1992	18	F	80.5	1.9058	0.410	0.59	22.90	22.49	75.9	23.49	76.77
Feb 25, 1992	19	F	56.0	1.7482	0.410	0.59	8.94	8.53	55.5	9.53	57.79
Feb 25, 1992	20	F	55.0	1.7404	0.410	0.59	8.51	8.10	54.4	9.10	56.82
Feb 25, 1992	21	F	55.0	1.7404	0.410	0.59	8.51	8.10	54.4	9.10	56.82

APPENDIX 6. Projected age and fork length data for wild-caught striped trumpeter, Latris lineata.

SAMPLING DATE	SAMPLE NUMBER	SEX	FORK LTH (cm)	Log10 F.L.	AGE ADJ. BACK	AGE ADJ. FORWARD	AGE AT SAMPLING	AGE PREV. SPAWN	F.L. PREV. SPAWN	AGE NEXT SPAWN	F.L. NEXT SPAWN
Feb 25, 1992	23	F	66.0	1.8195	0.410	0.59	14.01	13.60	65.3	14.60	66.76
Feb 25, 1992	24	F	52.0	1.7160	0.410	0.59	7.34	6.93	51.1	7.93	53.91
Feb 25, 1992	25	F	54.0	1.7324	0.410	0.59	8.10	7.69	53.3	8.69	55.85
Feb 25, 1992	29	F	61.0	1.7853	0.410	0.59	11.32	10.91	60.6	11.91	62.47
Feb 25, 1992	30	F	56.0	1.7482	0.410	0.59	8.94	8.53	55.5	9.53	57.79
Feb 25, 1992	31	F	53.0	1.7243	0.410	0.59	7.71	7.30	52.2	8.30	54.88
Feb 25, 1992	35	F	50.0	1.6990	0.410	0.59	6.66	6.25	48.9	7.25	52.03
Feb 25, 1992	37	F	57.0	1.7559	0.410	0.59	9.39	8.98	56.5	9.98	58.75
Feb 25, 1992	40	F	59.5	1.7745	0.410	0.59	10.57	10.16	59.1	11.16	61.11
Feb 25, 1992	2	M	51.5	1.7118	0.410	0.59	7.16	6.75	50.5	7.75	53.43
Feb 25, 1992	5	M	48.5	1.6857	0.410	0.59	6.22	5.81	47.4	6.81	50.70
Feb 25, 1992	7	M	42.5	1.6284	0.410	0.59	5.11	4.70	42.9	5.70	46.98
Feb 25, 1992	8	M	61.0	1.7853	0.410	0.59	11.32	10.91	60.6	11.91	62.47
Feb 25, 1992	9	M	53.5	1.7284	0.410	0.59	7.90	7.49	52.7	8.49	55.36
Feb 25, 1992	10	M	55.5	1.7443	0.410	0.59	8.73	8.32	54.9	9.32	57.31
Feb 25, 1992	13	M	55.0	1.7404	0.410	0.59	8.51	8.10	54.4	9.10	56.82
Feb 25, 1992	15	M	55.0	1.7404	0.410	0.59	8.51	8.10	54.4	9.10	56.82
Feb 25, 1992	16	M	58.0	1.7634	0.410	0.59	9.85	9.44	57.6	10.44	59.70
Feb 25, 1992	17	M	54.5	1.7364	0.410	0.59	8.31	7.90	53.8	8.90	56.34
Feb 25, 1992	22	M	56.0	1.7482	0.410	0.59	8.94	8.53	55.5	9.53	57.79
Feb 25, 1992	26	M	64.0	1.8062	0.410	0.59	12.90	12.49	63.5	13.49	65.10
Feb 25, 1992	27	M	55.0	1.7404	0.410	0.59	8.51	8.10	54.4	9.10	56.82
Feb 25, 1992	28	M	59.5	1.7745	0.410	0.59	10.57	10.16	59.1	11.16	61.11
Feb 25, 1992	32	M	56.5	1.7520	0.410	0.59	9.16	8.75	56.0	9.75	58.27
Feb 25, 1992	33	M	55.0	1.7404	0.410	0.59	8.51	8.10	54.4	9.10	56.82
Feb 25, 1992	34	M	57.5	1.7597	0.410	0.59	9.62	9.21	57.1	10.21	59.23
Feb 25, 1992	36	M	61.0	1.7853	0.410	0.59	11.32	10.91	60.6	11.91	62.47
Feb 25, 1992	38	M	59.0	1.7709	0.410	0.59	10.33	9.92	58.6	10.92	60.64
Feb 25, 1992	39	M	54.5	1.7364	0.410	0.59	8.31	7.90	53.8	8.90	56.34
May 22, 1992	1	F	66.5	1.8228	0.470	0.53	14.29	13.82	65.6	14.82	67.07
May 22, 1992	4	F	52.5	1.7202	0.470	0.53	7.52	7.05	51.4	8.05	54.24
May 22, 1992	7	F	57.0	1.7559	0.470	0.53	9.39	8.92	56.4	9.92	58.63
May 25, 1992	2	M	66.0	1.8195	0.640	0.36	14.01	13.37	64.9	14.37	66.42
May 25, 1992	3	M	52.5	1.7202	0.640	0.36	7.52	6.88	50.9	7.88	53.79
May 25, 1992	5	M	53.5	1.7284	0.640	0.36	7.90	7.26	52.1	8.26	54.79
May 25, 1992	6	M	56.5	1.7520	0.640	0.36	9.16	8.52	55.4	9.52	57.77
May 25, 1992	8	M	47.5	1.6767	0.640	0.36	5.95	5.31	45.5	6.31	49.12
May 25, 1992	9	M	53.5	1.7284	0.640	0.36	7.90	7.26	52.1	8.26	54.79
May 25, 1992	10	M	57.0	1.7559	0.640	0.36	9.39	8.75	56.0	9.75	58.26
May 25, 1992	11	M	63.0	1.7993	0.640	0.36	12.36	11.72	62.1	12.72	63.86

APPENDIX 7: Two-way ANOVA of the effects of sampling date and sex, on the gonad index recorded for *Latris lineata*.

Anova table for a 2-factor Analysis of Variance on Y_1: GONAD INDEX

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
SEX (A)	1	254.104	254.104	1.38	.2411
DATE (B)	9	105942.983	11771.443	63.908	.0001
AB	9	5541.559	615.729	3.343	.0006
Error	313	57652.849	184.194		

There were no missing cells found.

Page 1 of the AB Incidence table on Y_1: GONAD INDEX

	DATE:	MAY 25, ...	JUL 12, ...	SEP 20, ...	SEP 29, ...	MAR 14, ...	JUN 14, ...
SEX	M	10 2.405	13 20.542	7 41.251	28 47.057	25 2.408	9 2.701
	F	10 9.019	12 14.028	18 20.883	17 56.825	27 10.223	12 8.833
Totals:		20 5.712	25 17.416	25 26.586	45 50.747	52 6.466	21 6.205

Page 2 of the AB Incidence table on Y_1: GONAD INDEX

	DATE:	OCT 4, 1...	JAN 22, ...	FEB 25, 1...	MAY 22, ...	Totals:
SEX	M	8 59.214	31 2.977	20 2.554	8 2.257	159 16.46
	F	11 50.433	44 9.829	20 11.711	3 12.693	174 18.632
Totals:		19 54.13	75 6.997	40 7.132	11 5.104	333 17.595

APPENDIX 8. **Two sample t-tests for the effect of sex on gonad index recorded for *Latris lineata* for each sampling date.**

A. 25 MAY 1990

Unpaired t-Test X_1: SEX Y_1: GONAD INDEX

DF:	Unpaired t Value:	Prob. (1-tail):
18	-4.883	.0001

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
M	10	2.405	1.383	.437
F	10	9.019	4.054	1.282

B. 12 JULY 1990

Unpaired t-Test X_1: SEX Y_1: GONAD INDEX

DF:	Unpaired t Value:	Prob. (1-tail):
23	1.483	.0758

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
M	13	20.542	14.96	4.149
F	12	14.028	2.731	.789

C. 20 SEPTEMBER 1990

Unpaired t-Test X_1: SEX Y_1: GONAD INDEX

DF:	Unpaired t Value:	Prob. (1-tail):
23	2.319	.0148

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
M	7	41.251	26.668	10.08
F	18	20.883	16.586	3.909

D. 29 SEPTEMBER 1990

Unpaired t-Test X_1: SEX Y_1: GONAD INDEX

DF:	Unpaired t Value:	Prob. (1-tail):
43	-1.281	.1035

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
M	28	47.057	25.874	4.89
F	17	56.825	22.863	5.545

APPENDIX 8. (Cont.) **Two sample t-tests for the effect of sex on gonad index recorded for \ *Latris lineata* for each sampling date.**

E. 14 MARCH 1991

Unpaired t-Test X_1: SEX Y_1: GONAD INDEX

DF:	Unpaired t Value:	Prob. (1-tail):
50	-9.401	.0001

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
M	25	2.408	1.468	.294
F	27	10.223	3.907	.752

F. 14 JUNE 1991

Unpaired t-Test X_1: SEX Y_1: GONAD INDEX

DF:	Unpaired t Value:	Prob. (1-tail):
19	-3.976	.0004

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
M	9	2.701	1.756	.585
F	12	8.833	4.346	1.255

G. 4 OCTOBER 1991

Unpaired t-Test X_1: SEX Y_1: GONAD INDEX

DF:	Unpaired t Value:	Prob. (1-tail):
17	.583	.2838

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
M	8	59.214	28.735	10.159
F	11	50.433	34.768	10.483

H. 25 JANUARY 1992

Unpaired t-Test X_1: SEX Y_1: GONAD INDEX

DF:	Unpaired t Value:	Prob. (1-tail):
73	-11.416	.0001

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
M	31	2.977	1.562	.281
F	44	9.829	3.069	.463

APPENDIX 8. (Cont.) **Two sample t-tests for the effect of sex on gonad index recorded for \ *Latris lineata* for each sampling date.**

I. 25 FEBRUARY 1992

Unpaired t-Test X_1: SEX Y_1: GONAD INDEX

DF:	Unpaired t Value:	Prob. (1-tail):
38	-11.068	.0001

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
M	20	2.554	1.25	.28
F	20	11.711	3.482	.779

J. 22 MAY 1992

Unpaired t-Test X_1: SEX Y_1: GONAD INDEX

DF:	Unpaired t Value:	Prob. (1-tail):
9	-6.579	.0001

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
M	8	2.258	1.555	.55
F	3	12.693	4.03	2.327

APPENDIX 9A (Cont.)

One way ANOVA comparing the effect of date of sampling on the gonad index recorded for all male *Latris lineata* sampled (P = 0.05; N = 159).

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
JUL 12, 19... vs. FEB 25,...	17.988	10.159*	1.361	3.499
JUL 12, 19... vs. MAY 22...	18.285	12.814*	.884	2.82
SEP 20, 19... vs. SEP 29,...	-5.806	12.05	.101	.952
SEP 20, 19... vs. MAR 14...	38.844	12.194*	4.404*	6.295
SEP 20, 19... vs. JUN 14,...	38.55	14.37*	3.123*	5.301

* Significant at 95%

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
SEP 20, 19... vs. OCT 4, ...	-17.962	14.758*	.643	2.405
SEP 20, 19... vs. JAN 22...	38.275	11.933*	4.464*	6.339
SEP 20, 19... vs. FEB 25,...	38.697	12.523*	4.144*	6.107
SEP 20, 19... vs. MAY 22...	38.994	14.758*	3.029*	5.222
SEP 29, 19... vs. MAR 14...	44.65	7.846*	14.052*	11.246

* Significant at 95%

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
SEP 29, 19... vs. JUN 14,...	44.356	10.926*	7.151*	8.022
SEP 29, 19... vs. OCT 4, ...	-12.157	11.432*	.491	2.102
SEP 29, 19... vs. JAN 22...	44.08	7.434*	15.256*	11.717
SEP 29, 19... vs. FEB 25,...	44.503	8.348*	12.331*	10.535
SEP 29, 19... vs. MAY 22...	44.8	11.432*	6.664*	7.745

* Significant at 95%

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MAR 14, 1... vs. JUN 14,...	-.294	11.085	3.042E-4	.052
MAR 14, 1... vs. OCT 4, ...	-56.806	11.583*	10.437*	9.692
MAR 14, 1... vs. JAN 22,...	-.569	7.665	.002	.147
MAR 14, 1... vs. FEB 25, ...	-.146	8.555	1.271E-4	.034
MAR 14, 1... vs. MAY 22...	.15	11.583	7.287E-5	.026

* Significant at 95%

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
JUN 14, 19... vs. OCT 4, ...	-56.513	13.856*	7.218*	8.06
JUN 14, 19... vs. JAN 22...	-.276	10.797	2.829E-4	.05
JUN 14, 19... vs. FEB 25,...	.147	11.446	7.169E-5	.025
JUN 14, 19... vs. MAY 22...	.444	13.856	4.448E-4	.063
OCT 4, 1991 vs. JAN 22,...	56.237	11.308*	10.732*	9.828

* Significant at 95%

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
OCT 4, 1991 vs. FEB 25, ...	56.66	11.929*	9.79*	9.387
OCT 4, 1991 vs. MAY 22...	56.956	14.258*	6.925*	7.895
JAN 22, 1... vs. FEB 25, ...	.423	8.178	.001	.102
JAN 22, 1... vs. MAY 22,...	.719	11.308	.002	.126
FEB 25, 19... vs. MAY 22...	.296	11.929	2.681E-4	.049

* Significant at 95%

APPENDIX 9A: **One way ANOVA comparing the effect of date of sampling on the gonad index recorded for all male *Latris lineata* sampled (P = 0.05; N = 159).**

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	9	65088.088	7232.01	34.735
Within groups	149	31022.398	208.204	p = .0001
Total	158	96110.486		

Model II estimate of between component variance = 780.423

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
MAY 25, 1990	10	2.405	1.383	.437
JUL 12, 1990	13	20.542	14.96	4.149
SEP 20, 1990	7	41.251	26.668	10.08
SEP 29, 1990	28	47.057	25.874	4.89
MAR 14, 1991	25	2.408	1.468	.294

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
JUN 14, 1991	9	2.701	1.756	.585
OCT 4, 1991	8	59.214	28.735	10.159
JAN 22, 1992	31	2.977	1.562	.281
FEB 25, 1992	20	2.554	1.25	.28
MAY 22, 1992	8	2.258	1.555	.55

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MAY 25, 1... vs. JUL 12, ...	-18.137	11.994*	.992	2.988
MAY 25, 1... vs. SEP 20, ...	-38.846	14.052*	3.316*	5.463
MAY 25, 1... vs. SEP 29, ...	-44.652	10.505*	7.84*	8.4
MAY 25, 1... vs. MAR 14...	-.003	10.669	2.577E-8	4.816E-4
MAY 25, 1... vs. JUN 14,...	-.296	13.102	2.216E-4	.045

* Significant at 95%

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MAY 25, 1... vs. OCT 4, ...	-56.809	13.526*	7.654*	8.3
MAY 25, 1... vs. JAN 22,...	-.572	10.37	.001	.109
MAY 25, 1... vs. FEB 25, ...	-.149	11.044	7.899E-5	.027
MAY 25, 1... vs. MAY 22...	.148	13.526	5.160E-5	.022
JUL 12, 19... vs. SEP 20,...	-20.709	13.368*	1.041	3.061

* Significant at 95%

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
JUL 12, 19... vs. SEP 29,...	-26.515	9.57*	3.331*	5.475
JUL 12, 19... vs. MAR 14...	18.135	9.751*	1.501	3.675
JUL 12, 19... vs. JUN 14,...	17.841	12.365*	.903	2.851
JUL 12, 19... vs. OCT 4, ...	-38.671	12.814*	3.952*	5.964
JUL 12, 19... vs. JAN 22...	17.566	9.422*	1.508	3.684

* Significant at 95%

APPENDIX 9B: **One way ANOVA comparing the effect of date of sampling on the gonad index recorded for all female *Latris lineata* sampled (P = 0.05; N = 175).**

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	9	44727.299	4969.7	30.605
Within groups	164	26630.451	162.381	p = .0001
Total	173	71357.75		

Model II estimate of between component variance = 534.147

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
MAY 25, 1990	10	9.019	4.054	1.282
JUL 12, 1990	12	14.028	2.731	.789
SEP 20, 1990	18	20.883	16.586	3.909
SEP 29, 1990	17	56.825	22.863	5.545
MAR 14, 1991	27	10.223	3.907	.752

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
JUN 14, 1991	12	8.833	4.346	1.255
OCT 4, 1991	11	50.433	34.768	10.483
JAN 22, 1992	44	9.829	3.069	.463
FEB 25, 1992	20	11.711	3.482	.779
MAY 22, 1992	3	12.693	4.03	2.327

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MAY 25, 1... vs. JUL 12, ...	-5.009	10.774	.094	.918
MAY 25, 1... vs. SEP 20, ...	-11.864	9.925*	.619	2.361
MAY 25, 1... vs. SEP 29, ...	-47.806	10.028*	9.846*	9.414
MAY 25, 1... vs. MAR 14...	-1.204	9.315	.007	.255
MAY 25, 1... vs. JUN 14,...	.186	10.774	1.287E-4	.034

* Significant at 95%

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MAY 25, 1... vs. OCT 4, ...	-41.414	10.995*	6.147*	7.438
MAY 25, 1... vs. JAN 22,...	-.81	8.815	.004	.181
MAY 25, 1... vs. FEB 25, ...	-2.692	9.746	.033	.545
MAY 25, 1... vs. MAY 22...	-3.674	16.565	.021	.438
JUL 12, 19... vs. SEP 20,...	-6.855	9.378	.232	1.443

* Significant at 95%

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
JUL 12, 19... vs. SEP 29,...	-42.797	9.488*	8.816*	8.908
JUL 12, 19... vs. MAR 14...	3.805	8.73	.082	.861
JUL 12, 19... vs. JUN 14,...	5.195	10.273	.111	.999
JUL 12, 19... vs. OCT 4, ...	-36.404	10.504*	5.204*	6.844
JUL 12, 19... vs. JAN 22...	4.199	8.195	.114	1.012

* Significant at 95%

APPENDIX 9B: (Cont.) **One way ANOVA comparing the effect of date of sampling on the gonad index recorded for all female *Latris lineata* sampled (P = 0.05; N = 175).**

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
JUL 12, 19... vs. FEB 25,...	2.318	9.188	.028	.498
JUL 12, 19... vs. MAY 22...	1.335	16.243	.003	.162
SEP 20, 19... vs. SEP 29,...	-35.942	8.51*	7.728*	8.34
SEP 20, 19... vs. MAR 14...	10.66	7.657*	.84	2.749
SEP 20, 19... vs. JUN 14,...	12.05	9.378*	.715	2.537

* Significant at 95%

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
SEP 20, 19... vs. OCT 4, ...	-29.549	9.63*	4.079*	6.059
SEP 20, 19... vs. JAN 22...	11.054	7.041*	1.068	3.101
SEP 20, 19... vs. FEB 25,...	9.173	8.176*	.545	2.216
SEP 20, 19... vs. MAY 22...	8.19	15.692	.118	1.031
SEP 29, 19... vs. MAR 14...	46.602	7.791*	15.502*	11.812

* Significant at 95%

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
SEP 29, 19... vs. JUN 14,...	47.992	9.488*	11.086*	9.989
SEP 29, 19... vs. OCT 4, ...	6.393	9.737	.187	1.296
SEP 29, 19... vs. JAN 22...	46.996	7.186*	18.532*	12.915
SEP 29, 19... vs. FEB 25,...	45.115	8.301*	12.798*	10.732
SEP 29, 19... vs. MAY 22...	44.132	15.758*	3.398*	5.53

* Significant at 95%

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MAR 14, 1... vs. JUN 14,...	1.39	8.73	.011	.314
MAR 14, 1... vs. OCT 4, ...	-40.21	9.001*	8.647*	8.822
MAR 14, 1... vs. JAN 22,...	.394	6.152	.002	.127
MAR 14, 1... vs. FEB 25, ...	-1.488	7.424	.017	.396
MAR 14, 1... vs. MAY 22...	-2.47	15.314	.011	.319

* Significant at 95%

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
JUN 14, 19... vs. OCT 4, ...	-41.599	10.504*	6.796*	7.821
JUN 14, 19... vs. JAN 22...	-.996	8.195	.006	.24
JUN 14, 19... vs. FEB 25,...	-2.877	9.188	.042	.618
JUN 14, 19... vs. MAY 22...	-3.86	16.243	.024	.469
OCT 4, 1991 vs. JAN 22,...	40.604	8.483*	9.928*	9.452

* Significant at 95%

One Factor ANOVA X_1: DATE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
OCT 4, 1991 vs. FEB 25, ...	38.722	9.446*	7.281*	8.095
OCT 4, 1991 vs. MAY 22...	37.739	16.39*	2.297*	4.547
JAN 22, 1... vs. FEB 25, ...	-1.882	6.786	.033	.548
JAN 22, 1... vs. MAY 22,...	-2.864	15.015	.016	.377
FEB 25, 19... vs. MAY 22...	-.983	15.58	.002	.125

* Significant at 95%

APPENDIX 10 : Size frequency data for all Latris lineata sampled.

Bar:	SIZE INTERVAL (cm). From: (≥)	To: (<)	Count:	Percent:	
1	40	45	2	.599	
2	45	50	12	3.593	
3	50	55	101	30.24	
4	55	60	107	32.036	- Mode
5	60	65	55	16.467	
6	65	70	33	9.88	
7	70	75	11	3.293	
8	75	80	5	1.497	
9	80	85	7	2.096	
10	85	90	1	.299	

Frequency table of fork lengths (cm) for all *Latris lineata* sampled (N=334).

APPENDIX 11A: One way ANOVA of the effect of the maximum stage of gonad development, on the gonad index of male *Latris lineata* (P = 0.05).

One Factor ANOVA X_1: MAX. STAGE Y_1: GONAD INDEX

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	6	36202.68	6033.78	232.518
Within groups	84	2179.774	25.95	p = .0001
Total	90	38382.454		

Model II estimate of between component variance = 1001.305

One Factor ANOVA X_1: MAX. STAGE Y_1: GONAD INDEX

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
I	6	.46	.145	.059
DV	15	1.36	.482	.124
SR	34	3.249	1.401	.24
M1	16	3.639	2.204	.551
M2	7	18.721	7.908	2.989

One Factor ANOVA X_1: MAX. STAGE Y_1: GONAD INDEX

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
M	3	45.987	10.626	6.135
R	10	63.188	12.639	3.997

One Factor ANOVA X_1: MAX. STAGE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
I vs. DV	-.9	4.894	.022	.366
I vs. SR	-2.789	4.486	.255	1.237
I vs. M1	-3.179	4.85	.283	1.304
I vs. M2	-18.261	5.636*	6.92*	6.443
I vs. M	-45.527	7.164*	26.624*	12.639

* Significant at 95%

APPENDIX 11A (Cont.): **One way ANOVA of the effect of the maximum stage of gonad development, on the gonad index of male *Latris lineata* (P = 0.05).**

One Factor ANOVA X_1: MAX. STAGE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
DV vs. R	-61.828	4.136*	147.312*	29.73
SR vs. M1	-.39	3.071	.011	.253
SR vs. M2	-15.472	4.205*	8.925*	7.318
SR vs. M	-42.737	6.102*	32.339*	13.93
SR vs. R	-59.939	3.645*	178.302*	32.708

* Significant at 95%

One Factor ANOVA X_1: MAX. STAGE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
I vs. R	-62.728	5.232*	94.77*	23.846
DV vs. SR	-1.889	3.14	.239	1.197
DV vs. M1	-2.279	3.641	.258	1.245
DV vs. M2	-17.361	4.637*	9.24*	7.446
DV vs. M	-44.627	6.408*	31.978*	13.852

* Significant at 95%

One Factor ANOVA X_1: MAX. STAGE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
M1 vs. M2	-15.082	4.591*	7.114*	6.533
M1 vs. M	-42.347	6.374*	29.097*	13.213
M1 vs. R	-59.549	4.084*	140.154*	28.999
M2 vs. M	-27.265	6.991*	10.027*	7.756
M2 vs. R	-44.467	4.993*	52.292*	17.713

* Significant at 95%

One Factor ANOVA X_1: MAX. STAGE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
M vs. R	-17.201	6.669*	4.385*	5.13

* Significant at 95%

APPENDIX 11B: One way ANOVA of the effect of the maximum stage of oocyte development, on the gonad index of female *Latris lineata* (P = 0.05).

One Factor ANOVA X_1: MAX. STAGE Y_1: GONAD INDEX

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	5	32929.734	6585.947	81.009
Within groups	119	9674.569	81.299	p = .0001
Total	124	42604.303		

Model II estimate of between component variance = 1300.93

One Factor ANOVA X_1: MAX. STAGE Y_1: GONAD INDEX

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
PN	74	10.145	3.925	.456
CA	22	10.94	4.535	.967
VC	5	42.78	9.733	4.353
GVM	18	53.151	20.819	4.907
MAT	3	43.75	13.282	7.668

One Factor ANOVA X_1: MAX. STAGE Y_1: GONAD INDEX

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
NT	3	14.893	2.996	1.73

APPENDIX 11B (cont.): **One way ANOVA of the effect of the maximum stage of oocyte development, on the gonad index of female *Latris lineata* (P = 0.05).**

One Factor ANOVA X_1: MAX. STAGE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
PN vs. CA	-.795	4.336	.026	.363
PN vs. VC	-32.635	8.251*	12.271*	7.833
PN vs. GVM	-43.006	4.693*	65.875*	18.149
PN vs. MAT	-33.605	10.516*	8.01*	6.328
PN vs. NT	-4.748	10.516	.16	.894

* Significant at 95%

One Factor ANOVA X_1: MAX. STAGE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
CA vs. VC	-31.84	8.846*	10.161*	7.128
CA vs. GVM	-42.211	5.675*	43.394*	14.73
CA vs. MAT	-32.81	10.989*	6.991*	5.912
CA vs. NT	-3.953	10.989	.102	.712
VC vs. GVM	-10.371	9.026*	1.035	2.275

* Significant at 95%

One Factor ANOVA X_1: MAX. STAGE Y_1: GONAD INDEX

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
VC vs. MAT	-.97	13.04	.004	.147
VC vs. NT	27.887	13.04*	3.587*	4.235
GVM vs. MAT	9.401	11.135	.559	1.672
GVM vs. NT	38.258	11.135*	9.259*	6.804
MAT vs. NT	28.857	14.579*	3.073*	3.92

* Significant at 95%

APPENDIX 12: **One way ANOVA comparing the effect of the maximum stage of oocyte development category on the mean oocyte diameter (μm) of female *Latris lineata* (P = 0.05).**

One Factor ANOVA X_1: MAX. STAGE Y_1: MEAN MAX 10

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	5	5270482.167	1054096.433	572.992
Within groups	116	213397.619	1839.635	p = .0001
Total	121	5483879.787		

Model II estimate of between component variance = 210451.36

One Factor ANOVA X_1: MAX. STAGE Y_1: MEAN MAX 10

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
PN	72	127.121	14.918	1.758
CA	21	254.081	45.82	9.999
VC	5	605.26	36.638	16.385
GVM	18	622.139	38.041	8.966
MAT	3	849.367	30.094	17.375

One Factor ANOVA X_1: MAX. STAGE Y_1: MEAN MAX 10

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
NT	3	277.433	248.823	143.658

One Factor ANOVA X_1: MAX. STAGE Y_1: MEAN MAX 10

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
PN vs. CA	-126.96	21.071*	28.491*	11.935
PN vs. VC	-478.139	39.292*	116.203*	24.104
PN vs. GVM	-495.018	22.389*	383.622*	43.796
PN vs. MAT	-722.246	50.063*	163.328*	28.577
PN vs. NT	-150.313	50.063*	7.074*	5.947

* Significant at 95%

One Factor ANOVA X_1: MAX. STAGE Y_1: MEAN MAX 10

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
CA vs. VC	-351.179	42.277*	54.147*	16.454
CA vs. GVM	-368.058	27.29*	142.744*	26.716
CA vs. MAT	-595.286	52.438*	101.13*	22.487
CA vs. NT	-23.352	52.438	.156	.882
VC vs. GVM	-16.879	42.949	.121	.778

* Significant at 95%

One Factor ANOVA X_1: MAX. STAGE Y_1: MEAN MAX 10

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
VC vs. MAT	-244.107	62.046*	12.147*	7.793
VC vs. NT	327.827	62.046*	21.907*	10.466
GVM vs. MAT	-227.228	52.981*	14.434*	8.495
GVM vs. NT	344.706	52.981*	33.218*	12.888
MAT vs. NT	571.933	69.369*	53.343*	16.331

* Significant at 95%

APPENDIX 13 : Percentage and frequency of gonad development stage data for Latris lineata.

% MONTHLY SAMPLES OF FEMALE Latris lineata AT STAGE OF OOCYTE DEVELOPMENT.

SAMPLE	STAGE COUNT							% STAGE					
MONTH	PN	CA	VC	GVM	MAT	NT	N	PN	CA	VC	GVM	MAT	NT
Jan 22, 1992	18	0	0	0	0	0	18	100.0	0.0	0.0	0.0	0.0	0.0
Feb 25, 1992	19	0	0	0	0	0	19	100.0	0.0	0.0	0.0	0.0	0.0
Mar 14, 1991	13	0	0	0	0	0	13	100.0	0.0	0.0	0.0	0.0	0.0
May 22, 1992	1	2	0	0	0	0	3	33.3	66.7	0.0	0.0	0.0	0.0
May 25, 1990	10	0	0	0	0	0	10	100.0	0.0	0.0	0.0	0.0	0.0
MAY	11	2	0	0	0	0	13	84.6	15.4	0.0	0.0	0.0	0.0
Jun 14, 1991	10	2	0	0	0	0	12	83.3	16.7	0.0	0.0	0.0	0.0
Jul 12, 1990	2	10	0	0	0	0	12	16.7	83.3	0.0	0.0	0.0	0.0
Sep 20, 1990	1	8	3	5	0	0	17	5.9	47.1	17.6	29.4	0.0	0.0
Sep 29, 1990	0	0	0	12	3	1	16	0.0	0.0	0.0	75.0	18.8	6.3
SEP.	1	8	3	17	3	1	33	3.0	24.2	9.1	51.5	9.1	3.0
Oct 4, 1991	0	0	2	1	0	2	5	0.0	0.0	40.0	20.0	0.0	40.0
TOTALS	74	22	5	18	3	3	125						

% MONTHLY SAMPLES OF MALE Latris lineata AT STAGE OF GONAD DEVELOPMENT.

SAMPLE	STAGE COUNT							% STAGE					
MONTH	DV	SR	M1	M2	M	R	N	DV	SR	M1	M2	M	R
Jan 22, 1992	2	9	0	0	0	0	11	18.2	18.2	81.8	0.0	0.0	0.0
Feb 25, 1992	5	11	1	0	0	0	17	29.4	29.4	64.7	5.9	0.0	0.0
Mar 14, 1991	4	7	0	0	0	0	11	36.4	36.4	63.6	0.0	0.0	0.0
May 22, 1992	0	1	5	0	0	0	6	0.0	0.0	16.7	83.3	0.0	0.0
May 25, 1990	2	3	4	0	0	0	9	22.2	22.2	33.3	44.4	0.0	0.0
MAY	2	4	9	0	0	0	15	13.3	13.3	26.7	60.0	0.0	0.0
Jun 14, 1991	0	3	5	0	0	0	8	0.0	0.0	37.5	62.5	0.0	0.0
Jul 12, 1990	2	0	0	5	2	0	9	22.2	22.2	0.0	0.0	55.6	0.0
Sep 20, 1990	0	0	1	1	0	2	4	0.0	0.0	0.0	25.0	25.0	50.0
Sep 29, 1990	0	0	0	1	1	3	5	0.0	0.0	0.0	0.0	20.0	60.0
SEP.	0	0	1	2	1	5	9	0.0	0.0	0.0	11.1	22.2	55.6
Oct 4, 1991	0	0	0	0	0	5	5	0.0	0.0	0.0	0.0	0.0	100.0
TOTALS	15	34	16	7	3	10	85						

APPENDIX 14A: Presence of post-ovulatory follicles in Latris lineata during the spawning season.

SAMPLING DATE	GONAD INDEX	MEAN OF MAX. 10 OOCYTES (um).	MAX. STAGE OF DEVELOPMENT.	POST OVULATORY FOLLICLES (Yes/No).
Sep 20, 1990	52.2	579.6	GVM	N
Sep 20, 1990	4.5	290.5	CA	N
Sep 20, 1990	8.0	293.1	CA	N
Sep 20, 1990	8.6	311.6	CA	N
Sep 20, 1990	36.1	665.4	GVM	Y
Sep 20, 1990	5.4	302.1	CA	N
Sep 20, 1990	37.1	614.7	GVM	Y
Sep 20, 1990	33.6	681.8	GVM	Y
Sep 20, 1990	4.6	647.5	CA	N
Sep 20, 1990	34.9	647.5	GVM	Y
Sep 20, 1990	40.5	652.3	VC	Y
Sep 20, 1990	28.6	587.6	VC	N
Sep 20, 1990	5.0	211.2	CA	N
Sep 20, 1990	41.2	635.5	VC	Y
Sep 20, 1990	5.9	305.4	CA	N
Sep 20, 1990	5.8	178.9	PN	N
Sep 29, 1990	53.1	638.7	GVM	Y
Sep 29, 1990	54.9	700.6	GVM	N
Sep 29, 1990	84.6	598.8	GVM	Y
Sep 29, 1990	29.1	860.0	MAT	N
Sep 29, 1990	39.7	602.1	GVM	Y
Sep 29, 1990	51.6	629.1	GVM	Y
Sep 29, 1990	38.9	622.9	GVM	Y
Sep 29, 1990	55.0	815.4	MAT	Y
Sep 29, 1990	48.9	623.4	GVM	Y
Sep 29, 1990	39.7	557.6	GVM	Y
Sep 29, 1990	71.6	590.7	GVM	Y
Sep 29, 1990	25.8	573.5	GVM	Y
Sep 29, 1990	91.6	651.4	GVM	Y
Sep 29, 1990	53.7	584.2	VC	Y
Sep 29, 1990	47.1	872.7	MAT	N
Oct 4, 1991	18.3	564.7	VC	Y
Oct 4, 1991	95.0	627.4	GVM	N
Oct 4, 1991	49.9	566.7	VC	Y
Oct 4, 1991	13.9	138.4	PN	Y
Oct 4, 1991	12.5	129.2	PN	Y

APPENDIX 14B: Two-way ANOVA of the relationship between the maximum stage of oocyte development and the presence of post-ovulatory follicles, and the gonad index of female *Latris lineata*.

Anova table for a 2-factor Analysis of Variance on Y_1: GONAD INDEX

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
MAX. STAGE (A)	4	5450.444	1362.611	5.069	.0035
POST. OV FOLL'S (B)	1	825.654	825.654	3.072	.091
AB	4	108.668	27.167	.101	.9812
Error	27	7257.632	268.801		

There was 1 missing cell found.

The AB Incidence table on Y_1: GONAD INDEX

POST. OV FOL...		Y	N	Totals:
MAX. STAGE	PN	2 13.2	1 5.8	3 10.733
	CA	0 •	7 6	7 6
	VC	5 40.72	1 28.6	6 38.7
	GVM	14 49.086	3 67.367	17 52.312

Page 2 of the AB Incidence table on Y_1: GONAD INDEX

POST. OV FOL...		Y	N	Totals:
MA...	MAT	1 55	2 38.1	3 43.733
Totals:		22 44.191	14 25.336	36 36.858

APPENDIX 14C: Two-way ANOVA of the relationship between the maximum stage of oocyte development and the presence of post-ovulatory follicles, and the mean oocyte diameter of *Latris lineata*.

Anova table for a 2-factor Analysis of Variance on Y_1: MEAN MAX 10

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
MAX. STAGE (A)	4	683599.175	170899.794	30.948	.0001
POST. OV FOLL'S (B)	1	527.318	527.318	.095	.7597
AB	4	1603.741	400.935	.073	.9899
Error	27	149100.491	5522.24		

There was 1 missing cell found.

The AB Incidence table on Y_1: MEAN MAX 10

	POST. OV FOL...	Y	N	Totals:
MAX. STAGE	PN	2 133.8	1 178.9	3 148.833
	CA	0 •	7 337.343	7 337.343
	VC	5 600.68	1 587.6	6 598.5
	GVM	14 621.257	3 635.867	17 623.835

Page 2 of the AB Incidence table on Y_1: MEAN MAX 10

	POST. OV FOL...	Y	N	Totals:
MA...	MAT	1 815.4	2 866.35	3 849.367
Totals:		22 581.091	14 483.443	36 543.117

APPENDIX 15A: **Projected age and fork length (cm) of male *Latris lineata* at the time of next spawning, for immature (I), developing virgin (DV) and spent recovering (SR), maturity classes.**

X_1: I AGE NEXT

Mean:	Std. Dev.:	Std. Error:	Variance:	Coef. Var.:	Count:
7.265	1.709	.698	2.92	23.521	6
Minimum:	**Maximum:**	**Range:**	**Sum:**	**Sum Squared:**	**# Missing:**
5.7	10.09	4.39	43.59	331.282	123

X_2: I F.L. NEXT

Mean:	Std. Dev.:	Std. Error:	Variance:	Coef. Var.:	Count:
51.627	4.696	1.917	22.051	9.096	6
Minimum:	**Maximum:**	**Range:**	**Sum:**	**Sum Squared:**	**# Missing:**
46.98	58.98	12	309.76	16102.133	123

X_3: DV AGE NEXT

Mean:	Std. Dev.:	Std. Error:	Variance:	Coef. Var.:	Count:
9.189	1.142	.295	1.305	12.431	15
Minimum:	**Maximum:**	**Range:**	**Sum:**	**Sum Squared:**	**# Missing:**
7.75	11.79	4.04	137.83	1284.739	114

X_4: DV F.L. NEXT

Mean:	Std. Dev.:	Std. Error:	Variance:	Coef. Var.:	Count:
56.874	2.5	.645	6.249	4.395	15
Minimum:	**Maximum:**	**Range:**	**Sum:**	**Sum Squared:**	**# Missing:**
53.43	62.26	8.83	853.11	48607.261	114

X_5: SR AGE NEXT

Mean:	Std. Dev.:	Std. Error:	Variance:	Coef. Var.:	Count:
10.853	2.244	.385	5.036	20.677	34
Minimum:	**Maximum:**	**Range:**	**Sum:**	**Sum Squared:**	**# Missing:**
6.81	16.09	9.28	368.99	4170.697	95

X_6: SR F.L. NEXT

Mean:	Std. Dev.:	Std. Error:	Variance:	Coef. Var.:	Count:
60.07	4.45	.763	19.805	7.408	34
Minimum:	**Maximum:**	**Range:**	**Sum:**	**Sum Squared:**	**# Missing:**
50.7	68.8	18.1	2042.37	123338.118	95

APPENDIX 15B: **Projected age and fork length (cm) of female *Latris lineata* at the time of next spawning for developing virgin (DV) and spent recovering (SR), maturity classes.**

X_1: F/DV AGE NEXT

Mean:	Std. Dev.:	Std. Error:	Variance:	Coef. Var.:	Count:
6.952	1.213	.229	1.472	17.451	28
Minimum:	**Maximum:**	**Range:**	**Sum:**	**Sum Squared:**	**# Missing:**
4.9	9.31	4.41	194.67	1393.19	101

X_2: F/DV F.L. NEXT

Mean:	Std. Dev.:	Std. Error:	Variance:	Coef. Var.:	Count:
50.836	3.674	.694	13.497	7.227	28
Minimum:	**Maximum:**	**Range:**	**Sum:**	**Sum Squared:**	**# Missing:**
43.8	57.3	13.5	1423.4	72723.98	101

X_3: F/SR AGE NEXT

Mean:	Std. Dev.:	Std. Error:	Variance:	Coef. Var.:	Count:
11.741	4.754	.701	22.598	40.487	46
Minimum:	**Maximum:**	**Range:**	**Sum:**	**Sum Squared:**	**# Missing:**
6.51	25.46	18.95	540.1	7358.397	83

X_4: F/SR F.L. NEXT

Mean:	Std. Dev.:	Std. Error:	Variance:	Coef. Var.:	Count:
60.785	7.422	1.094	55.088	12.211	46
Minimum:	**Maximum:**	**Range:**	**Sum:**	**Sum Squared:**	**# Missing:**
49.8	78.5	28.7	2796.1	172439.31	83

APPENDIX 15C: One way ANOVA comparing the projected age of developing virgin (DV) male and female *Latris lineata* at the time of the next spawning season (P = 0.05).

One Factor ANOVA X_1: SEX Y_1: AGE NEXT SPAWN

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	48.842	48.842	34.519
Within groups	41	58.012	1.415	p = .0001
Total	42	106.853		

Model II estimate of between component variance = 47.427

One Factor ANOVA X_1: SEX Y_1: AGE NEXT SPAWN

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
M	15	9.189	1.142	.295
F	28	6.952	1.213	.229

One Factor ANOVA X_1: SEX Y_1: AGE NEXT SPAWN

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
M vs. F	2.236	.769*	34.519*	5.875

* Significant at 95%

APPENDIX 15D: **One way ANOVA comparing the projected fork length (cm) of developing virgin (DV) male and female *Latris lineata* at the time of the next spawning season (P = 0.05).**

One Factor ANOVA X_1: SEX Y_1: F.L. NEXT SPAWN

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	356.13	356.13	32.31
Within groups	41	451.907	11.022	p = .0001
Total	42	808.037		

Model II estimate of between component variance = 345.108

One Factor ANOVA X_1: SEX Y_1: F.L. NEXT SPAWN

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
M	15	56.874	2.5	.645
F	28	50.836	3.674	.694

One Factor ANOVA X_1: SEX Y_1: F.L. NEXT SPAWN

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
M vs. F	6.038	2.146*	32.31*	5.684

* Significant at 95%

APPENDIX 16A: Gonad index data for Latris lineata with/out atretic oocytes.

SAMPLING DATE	ATRETIC OOCYTES	GONAD INDEX	SAMPLING DATE	ATRETIC OOCYTES	GONAD INDEX
Feb 25, 1992	N	0.86	Jun 14, 1991	N	0.88
Feb 25, 1992	N	1.09	Jun 14, 1991	N	0.78
Feb 25, 1992	N	0.79	Jun 14, 1991	N	0.67
Feb 25, 1992	Y	1.29	Jun 14, 1991	N	0.72
Feb 25, 1992	Y	1.55	Jun 14, 1991	N	0.59
Feb 25, 1992	Y	1.36	Jun 14, 1991	N	0.87
Feb 25, 1992	Y	1.99	Jun 14, 1991	N	0.57
Feb 25, 1992	Y	1.80	Jun 14, 1991	N	0.78
Feb 25, 1992	Y	1.40	Jun 14, 1991	N	0.49
Feb 25, 1992	Y	1.02	Jun 14, 1991	N	0.70
Feb 25, 1992	Y	0.73	Jun 14, 1991	Y	1.70
Feb 25, 1992	Y	1.02	Jun 14, 1991	Y	1.86
Feb 25, 1992	Y	1.15	Mar 14, 1991	N	0.67
Feb 25, 1992	Y	0.76	Mar 14, 1991	N	0.70
Feb 25, 1992	Y	1.43	Mar 14, 1991	N	0.70
Feb 25, 1992	Y	1.01	Mar 14, 1991	N	0.65
Feb 25, 1992	Y	1.41	Mar 14, 1991	N	0.86
Feb 25, 1992	Y	0.92	Mar 14, 1991	N	0.72
Feb 25, 1992	Y	0.98	Mar 14, 1991	N	0.48
Jan 22, 1992	N	0.72	Mar 14, 1991	Y	1.30
Jan 22, 1992	N	1.11	Mar 14, 1991	Y	0.61
Jan 22, 1992	N	0.29	Mar 14, 1991	Y	1.59
Jan 22, 1992	N	0.74	Mar 14, 1991	Y	0.75
Jan 22, 1992	N	0.56	Mar 14, 1991	Y	1.43
Jan 22, 1992	Y	0.92	Mar 14, 1991	Y	1.05
Jan 22, 1992	Y	1.04	Mar 14, 1991	Y	1.40
Jan 22, 1992	Y	1.26	May 25, 1990	N	0.53
Jan 22, 1992	Y	0.76	May 25, 1990	N	0.47
Jan 22, 1992	Y	1.38	May 25, 1990	N	0.65
Jan 22, 1992	Y	1.44	May 25, 1990	Y	0.64
Jan 22, 1992	Y	0.91	May 25, 1990	Y	1.64
Jan 22, 1992	Y	1.33	May 25, 1990	Y	1.33
Jan 22, 1992	Y	1.14	May 25, 1990	Y	0.78
Jan 22, 1992	Y	1.76	May 25, 1990	Y	1.30
Jan 22, 1992	Y	0.72	May 25, 1990	Y	1.07
Jan 22, 1992	Y	0.71	May 25, 1990	Y	0.60
Jan 22, 1992	Y	1.12	May 25, 1990	Y	1.25

APPENDIX 16B: One way ANOVA of the effect of atretic oocytes being present/absent in the ovaries, on the gonad index of pre-vitellogenic *Latris lineata* (P = 0.05).

One Factor ANOVA X_1: ATRETIC Y_1: G.I.

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	410.166	410.166	44.489
Within groups	72	663.803	9.219	p = .0001
Total	73	1073.969		

Model II estimate of between component variance = 400.947

One Factor ANOVA X_1: ATRETIC Y_1: G.I.

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Y	46	11.87	3.585	.529
N	28	7.015	1.779	.336

One Factor ANOVA X_1: ATRETIC Y_1: G.I.

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Y vs. N	4.854	1.451*	44.489*	6.67

* Significant at 95%

APPENDIX 17: 1991 DSF HORMONE INDUCTION TRIAL RESULTS.

Mean oocyte diameter (µm)/fish/treatment, treatment means and change in mean oocyte diameter, at each 48 hr sampling interval.

					HOURS AFTER FIRST INJECTION/ MEAN OF MAX. 10 OOCYTES (um) +/- STANDARD ERROR								CHANGE IN MEAN O.D.		
	FISH	WEIGHT	TANK	INITIAL OOCYTE											
TREATMENT	NO.	(g)	NO.	CATEGORY	0 hr.	+/- S.E.	+48 hrs.	+/- S.E.	+96 hrs.	+/- S.E.	+144 hrs.	+/- S.E.	+48 hrs	+96 hrs	+144 hrs
OVAPRIM	#047	3600	1	S	360	0.0	307	26.7	222	2.0	230	4.5	-53	-138	-130
OVAPRIM	#033	2700	3	M	460	3.0	496	4.0	526	4.3	552	6.8	36	66	92
OVAPRIM	#022	4380	2	L	636	5.8	688	9.5	852	32.4	1190	5.4	52	216	554
Mean +/- S.E.					485.3	80.7	502.2	110.2	533.3	181.9	657.3	282.1	16.9	48	172
LHRHa(10ug)	#043	4000	3	S	410	6.8	518	2.0	544	2.7	574	5.2	108	134	164
LHRHa(10ug)	#044	4400	1	M	454	5.2	436	5.0	482	2.0	488	3.3	-18	28	34
LHRHa(10ug)	#054	2280	2	L	804	10.2	1006	8.7	1304	4.0	1320	7.3	202	500	516
Mean +/- S.E.					547.3	109.6	653.3	178.0	776.7	264.3	795.7	263.5	106.0	229.4	248.4
LHRHa(25ug)	#232	6000	3	S	400	27.5	332	10.4	286	3.1	306	7.3	-68	-114	-94
LHRHa(25ug)	#030	3500	3	L	562	2.0	634	5.2	666	3.1	686	9.9	72	104	124
LHRHa(25ug)	#000	3500	1	M	490	3.3	506	7.8	518	4.7	506	3.1	16	28	16
Mean +/- S.E.					480.7	46.7	501.7	77.7	490.0	110.6	498.7	109.7	21.0	9.3	18.0
													0	0	0
LHRHa(50ug)	#039	2700	2	S	442	3.6	468	3.3	472	3.3	472	4.4	26	30	30
LHRHa(50ug)	#060	2720	1	L	614	6.0	696	8.8	1214	13.7	1228	6.1	82	600	614
LHRHa(50ug)	#048	3370	1	M	492	7.4	464	2.7	480	5.2	478	4.7	-28	-12	-14
Mean +/- S.E.					517.3	50.8	542.7	76.7	722.0	246.0	726.0	251.0	25.4	204.7	208.7
CONTROL	#042	2280	2	S	432	9.5	360	9.4	382	5.5	390	6.8	-72	-50	-42
CONTROL	#903	3900	3	M	472	3.3	512	3.3	518	5.5	508	3.3	40	46	36
CONTROL	#064	2520	2	L	538	3.6	536	4.0	476	5.8	464	8.8	-2	-62	-74
Mean +/- S.E.					479.3	32.0	469.3	55.1	458.7	40.2	450.3	34.1	-10.0	-20.6	-29.0

APPENDIX 18a: 1991 DSF hormone induction trial.
Two factor repeated measures ANOVA of mean oocyte diameters (μm) for experimental treatments at each 48 hour sampling interval.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
TREAT. (A)	4	432541.963	108135.491	.447	.7725
subjects w. groups	10	2418637.248	241863.725		
Repeated Measure (B)	3	143695.658	47898.553	2.905	.0509
AB	12	147864.715	12322.06	.747	.696
B x subjects w. groups	30	494689.345	16489.645		

Repeated Mea...		0 HOURS	48 HOURS	96 HOURS	144 HOURS	Totals:
TREAT...	CONTROL	3 479.333	3 469.333	3 458.667	3 450.333	12 464.417
	OVAPRIM	3 485.333	3 502.233	3 533.333	3 657.333	12 544.558
	L10LHRHa	3 547.333	3 653.333	3 776.667	3 795.667	12 693.25
	M25LHRHa	3 480.667	3 501.667	3 490	3 498.667	12 492.75
TR...	H50LHRKa	3 517.333	3 542.667	3 722	3 726	12 627
Totals:		15 502	15 533.847	15 596.133	15 625.6	60 564.395

Appendix 18 b: DSF 1991 hormone induction trial.
One way ANOVA of the effect of experimental treatments on mean oocyte diameter at 48 hour intervals following the first injection.

1. +48 hours (time of the second injection)
2. +96 hours (time of the third injection/ 48 hr following injection 2)
3. +144 hours (48 hr after injection 3)

(1)

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	4	61655.344	15413.836	.437
Within groups	10	352462.593	35246.259	p = .7791
Total	14	414117.937		

Model II estimate of between component variance = -4958.106

(2)

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	4	247619.733	61904.933	.582
Within groups	10	1063776	106377.6	p = .6828
Total	14	1311395.733		

Model II estimate of between component variance = -11118.167

(3)

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	4	260520.933	65130.233	.482
Within groups	10	1351210.667	135121.067	p = .7489
Total	14	1611731.6		

Model II estimate of between component variance = -17497.708

Appendix 19: One way ANOVA of the total change in mean oocyte diameter after the 144 hr experimental period, for each experimental treatment.

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	4	171537.6	42884.4	.666
Within groups	10	643861.333	64386.133	p = .6299
Total	14	815398.933		

Model II estimate of between component variance = -5375.433

APPENDIX 20A: **DSF 1991 hormone induction trial.**
ANOVA - Effect of initial oocyte size category on change in mean oocyte diameter (μm) after 144 hr of treatment (P = 0.05).

Anova table for a 2-factor repeated measures Anova.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
OOC.SIZE CAT. (A)	2	1825176.303	912588.152	10.674	.0022
subjects w. groups	12	1026002.908	85500.242		
Repeated Measure (B)	3	143695.658	47898.553	4.808	.0065
AB	6	283878.456	47313.076	4.749	.0012
B x subjects w. groups	36	358675.604	9963.211		

There were no missing cells found.

The AB Incidence table

Repeated Mea...		0 HOURS	48 HOURS	96 HOURS	144 HOURS	Totals:
OOC.SIZE CAT.	SMALL	5 430.4	5 386.34	5 370.4	5 378.4	20 391.385
	MEDIUM	5 452.8	5 503.2	5 515.6	5 523	20 498.65
	LARGE	5 622.8	5 712	5 902.4	5 975.4	20 803.15
Totals:		15 502	15 533.847	15 596.133	15 625.6	60 564.395

APPENDIX 20B: **DSF 1991 hormone induction trial.**
One way ANOVA (P = 0.05) of the 144 hr change in mean oocyte diameter for *latris lineata* with different initial oocyte size categories (small, medium large) following commencement of spawning induction.

One Factor ANOVA X_1: OOC.SIZE CAT. Y_1: CHANGE O.D.

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	2	414018.133	207009.067	6.189
Within groups	12	401380.8	33448.4	p = .0142
Total	14	815398.933		

Model II estimate of between component variance = 86780.333

One Factor ANOVA X_1: OOC.SIZE CAT. Y_1: CHANGE O.D.

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
SMALL	5	-49.2	64.709	28.939
MEDIUM	5	67.6	61.162	27.353
LARGE	5	346.8	304.002	135.954

One Factor ANOVA X_1: OOC.SIZE CAT. Y_1: CHANGE O.D.

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
SMALL vs. MEDIUM	-116.8	252.05	.51	1.01
SMALL vs. LARGE	-396	252.05*	5.86*	3.424
MEDIUM vs. LARGE	-279.2	252.05*	2.913	2.414

* Significant at 95%

APPENDIX 21. Constituents of sustained release hormone implants used in 1992 spawning induction trials. (Amounts to make up 500 mg of pellet mix.)

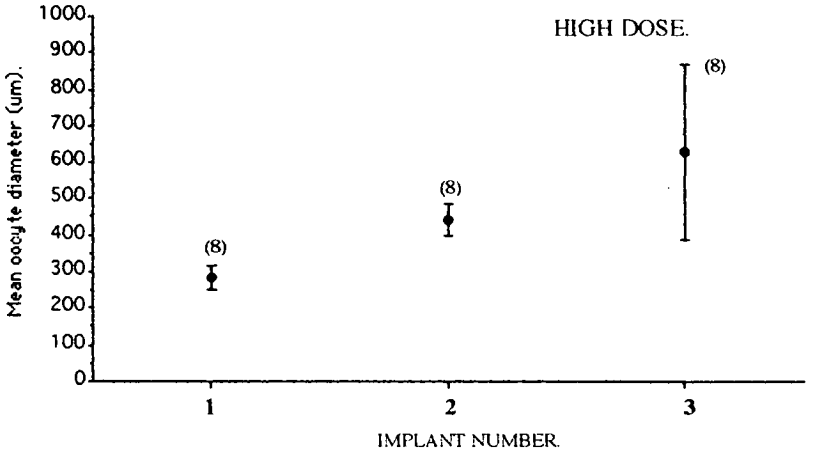
	DOSE RANGE (EXPERIMENT)/CONTENTS (mg).		
IMPLANT NO.	HIGH (DSF/UTL)		LOW (DSF)
1	LHRHa stock	50	25
	17α-MT	200	100
	Extra cholesterol	202	327
	Coconut oil	48	48
2 and **3**.	LHRHa stock	200	100
	17α-MT	200	100
	Extra cholesterol	52	252
	Coconut oil	48	48

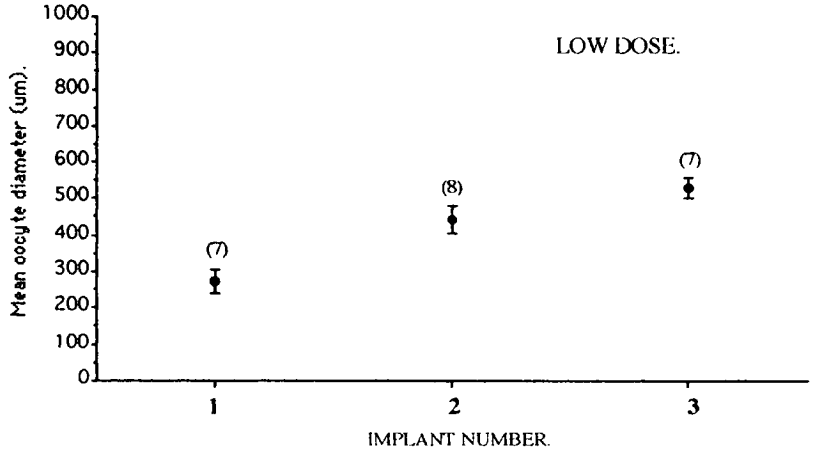
APPENDIX 22A: DSF 1992/ Mean oocyte diameter (um) of Latris lineata at each monthly implant.

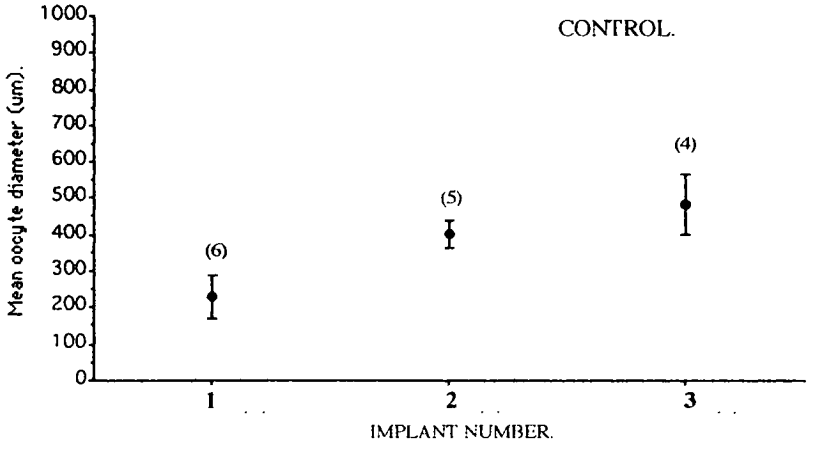
	MEAN DIAMETER (um) OF MAX. 10 OOCYTES.					
	IMPLANT 1.		IMPLANT 2.		IMPLANT 3.	
FISH NO TREATMEN	MEAN	+/- S.E.	MEAN	+/- S.E	MEAN	+/- S.E.
136 HIGH	292.9	8.6	426.3	5.7	532.0	9.1
141 HIGH	328.3	5.1	525.1	5.6	1210.6	5.7
146 HIGH	305.0	4.7	446.7	3.4	535.4	5.2
147 HIGH	302.0	3.8	463.8	5.6	610.4	3.4
148 HIGH	305.0	7.2	422.8	5.6	504.7	11.1
150 HIGH	254.5	3.9	419.4	5.2	538.8	8.5
153 HIGH	256.5	8.1	450.1	4.5	576.3	16.4
156 HIGH	225.2	3.0	378.5	3.4	497.9	10.4
TOTAL	283.8	12.2	441.6	15.0	625.9	84.6
142 LOW	250.5	4.7	450.1	6.8	508.1	24.1
143 LOW	N/A	-	399.0	10.2	494.5	17.1
144 LOW	255.5	6.9	463.8	5.6	525.1	5.6
145 LOW	314.1	4.4	480.8	3.4	555.8	5.2
149 LOW	267.7	5.5	371.7	10.7	Mort.	-
151 LOW	291.9	7.3	487.6	5.2	552.4	6.8
152 LOW	303.0	4.0	453.5	5.2	559.2	5.6
154 LOW	223.2	7.1	416.0	4.5	532.0	5.6
TOTAL	272.3	12.2	440.0	14.5	532.3	9.5
135 CONTROL	321.2	10.4	449.8	4.5	Mort.	-
137 CONTROL	N/A	-	N/A	-	N/A	-
138 CONTROL	232.3	4.0	426.3	7.6	480.8	14.8
139 CONTROL	234.0	5.2	Mort.	-	-	-
140 CONTROL	253.5	3.2	419.4	5.2	600.2	5.6
155 CONTROL	134.3	2.2	347.9	13.3	416.0	4.5
157 CONTROL	212.1	3.0	388.7	5.6	450.1	4.5
TOTAL	231.2	24.8	406.4	17.5	486.8	40.0

N/A = No sample by biopsy.
Mort. = Mortality.

APPENDIX 22B: **DSF 1992 sustained release hormone implant experiment. Mean oocyte diameter (μm) of *Latris lineata* for each experimental treatment, for each implant (1 =July; 2 = August; 3 = September).**
(Error bars = +/- standard deviation.)







APPENDIX 23A: DSF IMPLANT EXPERIMENT 1992.

Two way ANOVA comparing the effect of treatment and implant number (month) on mean oocyte diameter (μm) of *Latris lineata* (P = 0.05).

Anova table for a 2-factor Analysis of Variance on Y_1: OOC DIAM.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
IMPLANT (A)	2	790076.067	395038.033	42.187	.0001
TREAT. (B)	2	52575.49	26287.745	2.807	.0695
AB	4	26257.947	6564.487	.701	.5948
Error	52	486931.89	9364.075		

There were no missing cells found.

The AB Incidence table on Y_1: OOC DIAM.

	TREAT.:	HIGH	LOW	PLAC.	Totals:
IMPLANT	A	8 283.75	7 272.286	6 231.167	21 264.905
	B	8 441.625	8 440.5	5 406.4	21 432.81
	C	8 625.875	7 532.286	4 486.75	19 562.105
Totals:		24 450.417	22 416.182	15 357.733	61 415.279

APPENDIX 23B: DSF IMPLANT EXPERIMENT 1992.

One way ANOVA comparing the effect of implant number (month) on mean oocyte diameter (μm) of (P = 0.05) *Latris lineata*.

One Factor ANOVA X_1: IMPLANT Y_1: OOC DIAM.

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	2	890915.425	445457.713	45.888
Within groups	58	563036.837	9707.532	p = .0001
Total	60	1453952.262		

Model II estimate of between component variance = 217875.09

One Factor ANOVA X_1: IMPLANT Y_1: OOC DIAM.

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
A	21	264.905	46.377	10.12
B	21	432.81	41.864	9.135
C	19	562.105	164.143	37.657

One Factor ANOVA X_1: IMPLANT Y_1: OOC DIAM.

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
A vs. B	-167.905	60.87*	15.247*	5.522
A vs. C	-297.201	62.452*	45.381*	9.527
B vs. C	-129.296	62.452*	8.589*	4.145

* Significant at 95%

APPENDIX 24 A: DSF 1992/ Latris lineata serum 17b-estradiol data from RIA.

IMPLANT 1			14/7/92				15/7/92			
			0 Hours				+ 24 hours			
			Mean	Mean			Mean	Mean		
			estradiol	estradiol		QEH	estradiol	estradiol		QEH
TREAT.	FISH NO.	SEX	NM/L	ng/ml	% error	NM/L	NM/L	ng/ml	% error	NM/L
C	135	F	5.89	1.61	3.8	1.24	3.02	0.82	27.6	0.20
H	136	F	1.92	0.52	7.7	0.24				
C	137	F	0.65	0.18	5.8	0.29				
C	138	F	1.72	0.47	5.8	0.76	1.41	0.38	22.7	0.44
C	139	F	3.02	0.82	7.9	0.28	2.60	0.71	12.7	
C	140	F	4.79	1.31	4.9	1.13	4.29	1.17	22.1	
H	141	F	9.02	2.46	4.6	1.15	1.77	0.48	10.0	0.69
L	142	F	5.55	1.51	10.3	1.84				
L	143	F	3.85	1.05	5.6	0.53	1.81	0.49	28.0	0.46
L	144	F	2.39	0.65	6.4	0.48	0.34	0.09	24.9	0.83
L	145	F	0.47	0.13	5.9	0.12				
H	146	F	2.94	0.80	9.0	1.19	2.02	0.55	6.1	0.44
H	147	F	3.36	0.91	3.9	0.16				
H	148	F	2.98	0.81	3.8	0.77	0.64	0.17	0.5	0.40
L	149	F	3.63	0.99	0.9	0.64				
H	150	F	2.85	0.78	1.4	0.50				
L	151	F	1.54	0.42	0.5	0.44	0.34	0.09	9.0	0.59
L	152	F	1.89	0.51	7.3	0.53	0.88	0.24	5.3	
H	153	F	1.51	0.41	6.5	0.48	0.85	0.23	10.8	0.36
L	154	F	0.82	0.22	2.0	0.33				
C	155	F	0.70	0.19	1.2	0.23				
H	156	F	0.95	0.26	22.0	0.28				
C	157	F	1.35	0.37	7.5					
C	414	M	0.76	0.21	1.5	0.10	0.33	0.09	4.1	0.44
C	415	M	0.84	0.23	0.2	0.17	0.52	0.14	29.5	0.33
C	416	M	0.81	0.22	4.8	0.20	0.51	0.14	0.9	
C	417	M	0.63	0.17	10.9	0.32				
L	418	M	0.77	0.21	9.7	0.41	0.53	0.14	4.2	0.22
L	419	M	0.87	0.24	4.4	1.92	0.49	0.13	6.2	0.16
L	420	M	0.44	0.12	86.3	0.30				
L	421	M	0.64	0.18	2.9	0.60				
H	422	M	0.50	0.14	1.7	0.39	0.44	0.12	9.4	INSUFF
H	423	M	0.65	0.18	15.2	0.35				
H	424	M	0.70	0.19	2.6	0.13				
H	425	M	0.54	0.15	11.2	<0.07				

APPENDIX 24 A: DSF 1992/ Latris lineata serum 17b-estradiol data from RIA.

IMPLANT 2			11/8/92				12/8/92			
			0 Hours				+ 24 hours			
			Mean	Mean			Mean	Mean		
			estradiol	estradiol	% Error	QEH	estradiol	estradiol	% Error	QEH
C	135	F				1.09				
H	136	F				0.24				
C	137	F				0.30	0.54	0.15	28.9	0.15
C	138	F				0.88	2.09	0.57	17.2	0.42
C	139	F	Mortality.							
C	140	F				1.06				
H	141	F				1.20	17.88	4.87	6.6	1.18
L	142	F				1.15	0.30	0.08	13.3	0.68
L	143	F				0.30	1.15	0.31	21.0	0.30
L	144	F				0.43	0.68	0.19	34.9	0.15
L	145	F				2.94				
H	146	F				1.40	2.27	0.62	7.9	0.27
H	147	F				1.95				
H	148	F				0.86				
L	149	F	0.76	0.21	5.6	0.20				
H	150	F	2.53	0.69	4.5	0.76				
L	151	F	2.55	0.69	10.6	0.33				
L	152	F	3.51	0.96	1.6	1.19				
H	153	F	11.81	3.22	6.6	0.54	2.23	0.61	30.1	
L	154	F	4.98	1.36	0.6	1.52	2.19	0.60	25.5	0.47
C	155	F	1.36	0.37	11.7	0.58				
H	156	F	3.60	0.98	0.7	1.32	1.91	0.52	8.6	0.34
C	157	F	1.35	0.37	14.7	0.14	1.21	0.33	0.7	0.30
C	414	M	1.06	0.29	19.5	0.29				
C	415	M	0.89	0.24	9.3	<0.07	0.81	0.22	26.0	0.13
C	416	M	1.58	0.43	9.6	0.27				
C	417	M	0.58	0.16	5.5	0.10	0.45	0.12	15.0	
L	418	M	1.07	0.29	12.8	0.22				
L	419	M	0.42	0.11	10.7	0.17	0.51	0.14	7.9	0.33
L	420	M	0.96	0.26	11.6	0.11	0.55	0.15	10.8	0.19
L	421	M	Mortality.							
H	422	M	0.35	0.10	5.7	0.13	0.38	0.10	8.5	0.15
H	423	M	0.53	0.14	11.9	0.19				
H	424	M	1.25	0.34	4.5	0.12				
H	425	M	0.78	0.21	10.7	0.11				

APPENDIX 24 A: DSF 1992/ Latris lineata serum 17b-estradiol data from RIA.

IMPLANT 3			8/9/92				9/9/92			
			0 Hours				+ 24 hours			
			Mean	Mean			Mean	Mean		
			estradiol	estradiol	% error	QEH	estradiol	estradiol	% error	QEH
C	135	F	Mortality.							
H	136	F	0.38	0.10	7.0	0.16	0.52	0.14	5.7	0.21
C	137	F	0.61	0.17	22.2	0.26				
C	138	F	5.49	1.49	5.8	0.17	4.49	1.22	10.5	0.34
C	139	F	Mortality.							
C	140	F	21.28	5.80	23.3	1.57	17.77	4.84	2.5	1.82
H	141	F	7.52	2.05	16.8	1.14	8.28	2.26	1.6	2.36
L	142	F	0.40	0.11	1.0	0.14	0.47	0.13	4.2	INSUFF
L	143	F	5.10	1.39	10.3	0.51				
L	144	F	6.29	1.71	14.0	0.93	6.10	1.66	11.2	1.31
L	145	F	13.88	3.78	16.6	1.49				
H	146	F	5.58	1.52	18.5	0.74				
H	147	F	14.05	3.83	0.0	0.85				
H	148	F	5.79	1.58	5.5	0.54	4.23	1.15	14.6	0.59
L	149	F	Mortality.							
H	150	F	11.64	3.17	18.0	1.72	14.19	3.86	0.9	2.50
L	151	F	3.05	0.83	11.5	0.61				
L	152	F	6.52	1.78	15.2	1.91	1.91	0.52	1.8	1.06
H	153	F	8.04	2.19	0.0	0.66				
L	154	F	5.61	1.53	14.1	1.32	4.66	1.27	7.5	0.81
C	155	F	3.46	0.94	13.0	1.02	3.01	0.82	10.0	1.03
H	156	F	5.94	1.62	6.8	1.10				
C	157	F	10.36	2.82	4.1	1.88	11.15	3.04	7.8	2.88
								0.00		
C	414	M	0.79	0.22	8.2	0.30	0.95	0.26	26.8	0.23
C	415	M	0.83	0.23	18.6	0.08				
C	416	M	1.23	0.33	7.4	0.34	1.26	0.34	12.9	0.28
C	417	M	0.57	0.16	13.4	0.19				
L	418	M	1.42	0.39	25.7	0.37	0.97	0.26	12.9	INSUFF
L	419	M	Mortality.							
L	420	M	0.89	0.24	2.2	0.25	0.69	0.19	40.5	0.20
L	421	M	Mortality.							
H	422	M	0.59	0.16	3.9	0.21	0.60	0.16	16.2	0.23
H	423	M	1.22	0.33	15.7	0.23	0.81	0.22	4.7	0.31
H	424	M	0.73	0.20	19.0	0.18				
H	425	M	1.04	0.28	7.7	0.21				

APPENDIX 24B: DSF 1992/Latris lineata serum testosterone from RIA..

IMPLANT 1										
			14/7/92				15/7/92			
			0 Hours				+ 24 hours			
			Mean	Mean	% error		Mean	Mean	% error	
			Testost.	Testost.		QEH	Testost.	Testost.		QEH
TREAT.	FISH NO.	SEX	NM/L	ng/ml		NM/L	NM/L	ng/ml		NM/L
C	135	F	2.010	0.579	3.2		3.766	1.085	2.3	
H	136	F	0.508	0.146	7.4					
C	137	F	2.138	0.616	31.5					
C	138	F	0.996	0.287	22.0		2.069	0.596	1.6	
C	139	F	1.883	0.542	8.1		4.918	1.416	13.9	
C	140	F	2.003	0.577	15.6		5.110	1.472	3.8	
H	141	F	2.896	0.834	5.7		0.320	0.092	10.3	
L	142	F	3.611	1.040	6.9					
L	143	F	2.760	0.795	6.3		0.305	0.088	18.2	
L	144	F	1.654	0.476	5.1		3.308	0.953	3.3	
L	145	F	1.342	0.386	12.6					
H	146	F	0.493	0.142	0.0		0.122	0.035	82.2	
H	147	F	1.432	0.412	14.3					
H	148	F	1.206	0.347	18.3		OUT			
L	149	F	4.058	1.169	6.2					
H	150	F	1.081	0.311	9.4					
L	151	F	0.697	0.201	19.5		3.777	1.088	7.3	
L	152	F	0.725	0.209	1.4		3.589	1.034	1.8	
H	153	F	1.515	0.436	8.5		0.086	0.025	0.0	
L	154	F	0.322	0.093	18.2					
C	155	F	OUT	0.000						
H	156	F	0.346	0.100	10.4					
C	157	F	0.864	0.249	1.9					
C	414	M	2.051	0.591	5.9					
C	415	M	2.436	0.702	7.7					
C	416	M	3.697	1.065	6.9		2.864	0.825	4.0	
C	417	M	2.373	0.683	0.5					
L	418	M	2.224	0.641	12.5		1.381	0.398	7.9	
L	419	M	1.530	0.441	14.5		1.501	0.432	12.8	
L	420	M	2.477	0.713	14.0					
L	421	M	3.042	0.876	7.2					
H	422	M	1.808	0.521	6.1		1.764	0.508	7.9	
H	423	M	1.601	0.461	4.9					
H	424	M	2.558	0.737	8.8					
H	425	M	2.490	0.717	7.3					

APPENDIX 24B: DSF 1992/Latris lineata serum testosterone from RIA..

IMPLANT 2										
			11/8/92				12/8/92			
			0 Hours				+ 24 hours			
			Mean	Mean	% error		Mean	Mean	% error	
			Testost.	Testost.		QEH	Testost.	Testost.		QEH
TREAT.	FISH NO.	SEX	pmol/L	ng/ml		NM/L	pmol/L	ng/ml		NM/L
C	135	F	270.615		7.2	6.00				
H	136	F	OUT			3.10				
C	137	F	0.569		0.0	4.80	2.490		16.0	2.10
C	138	F	1.131		16.3	5.70	2.457		8.4	18.30
C	139	F								
C	140	F	OUT			1.90				
H	141	F	2.939		10.4	1.20	7.149		6.1	41.80
L	142	F	9.518		2.8	61.20	0.937		9.5	14.30
L	143	F	OUT			39.30	OUT			1.30
L	144	F	OUT			2.40	OUT			1.20
L	145	F	0.840		3.8	39.00				
H	146	F	OUT			23.80	2.025		31.9	27.60
H	147	F	8.585		0.1	74.30				
H	148	F	OUT			1.00				
L	149	F	OUT			1.00				
H	150	F	1.268		14.8	9.00				
L	151	F	1.075		6.7	11.00				
L	152	F	OUT			1.00				
H	153	F	12.233		24.5	40.00	OUT			
L	154	F	70.827		1.7	91.90	0.264		62.0	5.70
C	155	F	35.495		16.8	14.80				
H	156	F	15.299		2.7	6.10	OUT			1.10
C	157	F	8.049		3.3	0.60	OUT			1.50
C	414	M				3.60				
C	415	M				11.80	1.871		4.4	3.30
C	416	M				69.30				
C	417	M	OUT			2.20	2.125		10.3	
L	418	M	OUT			9.70				
L	419	M	OUT			6.30	6.409		1.4	23.30
L	420	M	43.747		12.5	70.70	0.741		9.0	1.60
L	421	M								
H	422	M	OUT			20.10	0.520		3.4	1.00
H	423	M	OUT			1.00	0.547		0.0	
H	424	M	3.761		1.2	16.00				
H	425	M	OUT			1.70				

APPENDIX 24B: DSF 1992/Latris lineata serum testosterone from RIA..

IMPLANT 3										
			8/9/92				9/9/92			
			0 Hours				+ 24 hours			
			Mean	Mean	% error		Mean	Mean	% error	
			Testost.	Testost.		QEH	Testost.	Testost.		QEH
TREAT.	FISH NO.	SEX	pmol/L	ng/ml		NM/L	pmol/L	ng/ml		NM/L
C	135	F								
H	136	F	OUT			1.40	OUT			1.2
C	137	F	1.461	0.421	16.4	1.10				
C	138	F	0.084	0.024	0.0	1.00	OUT			1.4
C	139	F								
C	140	F	3.829	1.103	0.6	53.90	3.974	1.145	10.1	2.9
H	141	F	OUT			0.80	OUT			1.8
L	142	F	OUT			1.60	OUT			1.6
L	143	F	OUT			1.50				
L	144	F	OUT			1.20	OUT			1.2
L	145	F	OUT			1.40				
H	146	F	OUT			1.20				
H	147	F	0.717	0.206	5.8	10.20				
H	148	F	0.866	0.249	21.4	3.80	OUT			1.8
L	149	F								
H	150	F	OUT			1.50	OUT			1.3
L	151	F	OUT			1.90				
L	152	F	OUT			1.30	OUT			1.6
H	153	F	OUT			0.80				
L	154	F	OUT			1.40	OUT			0.8
C	155	F	0.274	0.079	13.4	1.10	OUT			15.6
H	156	F	OUT			0.80				
C	157	F	1.721	0.496	22.7	1.40	OUT			1.4
C	414	M	1.670	0.481	8.8	1.80	1.799	0.518	5.8	2.6
C	415	M	2.116	0.609	1.3	29.00				
C	416	M	10.933	3.149	0.0	3.70	9.521	2.742	0.4	2.5
C	417	M	1.495	0.431	37.6	2.30				
L	418	M	2.814	0.810	10.3	3.90	2.372	0.683	15.8	2.2
L	419	M								
L	420	M	0.995	0.287	0.9	1.30	0.657	0.189	0.0	1.2
L	421	M								
H	422	M	1.069	0.308	4.0	1.40	0.432	0.124	18.3	1.5
H	423	M	0.689	0.198	2.2	1.50	0.707	0.204	10.5	3.3
H	424	M	1.015	0.292	0.5	0.90				
H	425	M	2.400	0.691	0.4	4.90				

APPENDIX 25: DSF 1992/Statistics for Latris lineata serum 17b-estradiol and testosterone data.

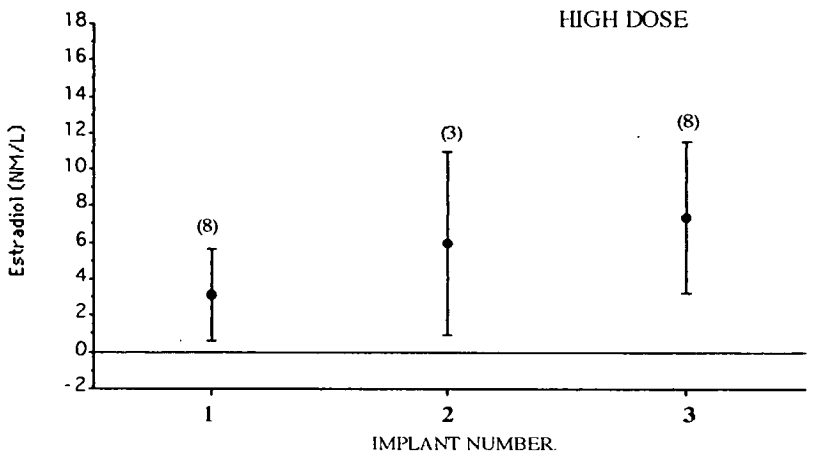
17 b-Estradiol (NM/L)

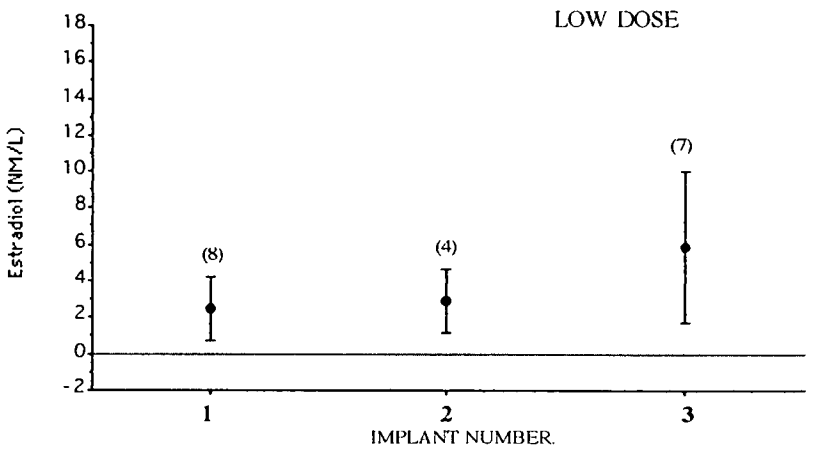
IMPLANT		Initial (0 hr)			+24 hr		
NUMBER	TREATMENT	Mean	Variance	n	Mean	Variance	n
1	HIGH	3.19	6.24	8	1.32	0.46	4
	LOW	2.52	2.95	8	0.84	0.48	4
	CONTROL	2.59	4.26	7	2.77	2.09	3
2	HIGH	5.98	25.78	3	6.07	61.70	4
	LOW	2.95	3.13	4	1.08	0.67	4
	CONTROL	1.36	0.00	2	1.28	0.60	3
3	HIGH	7.37	17.13	8	6.81	34.28	4
	LOW	5.84	17.21	7	3.28	6.54	4
	CONTROL	8.24	65.78	5	9.10	45.90	4

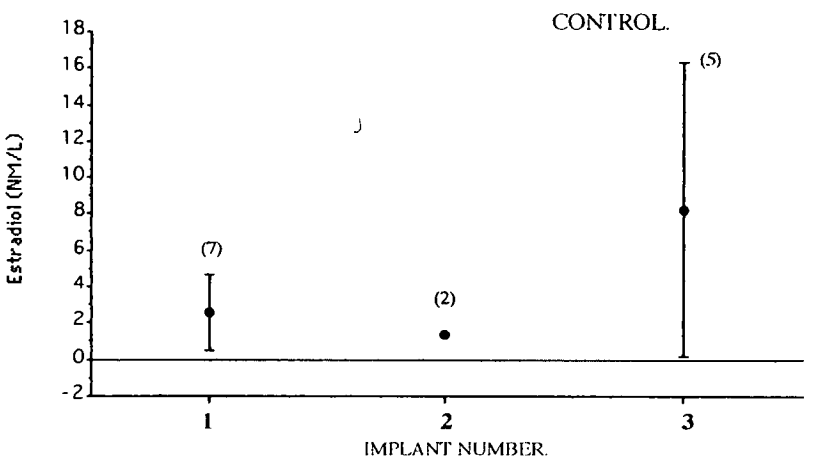
Testosterone (NM/L)

IMPLANT		Initial (0 hr)			+24 hr		
NUMBER	TREATMENT	Mean	Variance	n	Mean	Variance	n
1	HIGH	1.19	0.68	8	0.18	0.02	3
	LOW	1.88	2.03	8	2.75	59.77	4
	CONTROL	1.65	0.32	6	3.97	35.22	4
2	HIGH	19.81	668.13	8	23.50	87.90	3
	LOW	30.85	1110.22	8	5.63	109.37	4
	CONTROL	5.63	24.83	6	7.30	130.56	3
3	HIGH	2.56	10.5	8	1.53	20.99	4
	LOW	1.47	0.05	7	1.30	29.46	4
	CONTROL	11.70	556.54	5	5.33	129.32	4

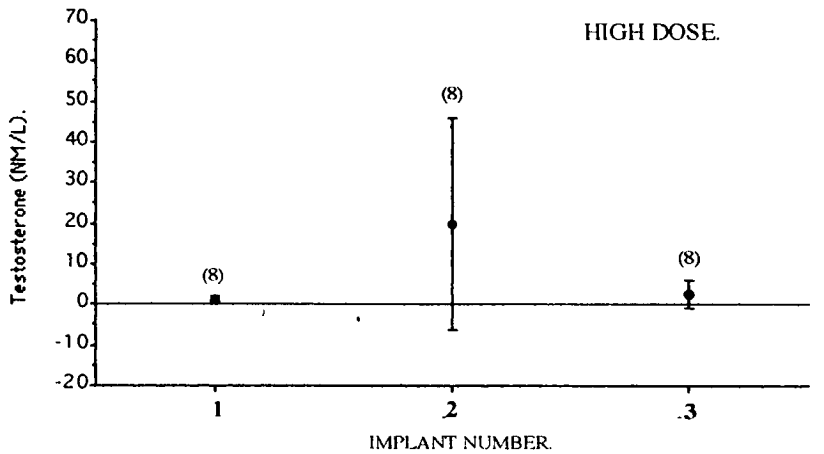
APPENDIX 26A: **DSF 1992 sustained release hormone implant experiment. Mean serum 17β-estradiol of *Latris lineata* for each experimental treatment, for each implant; 1 =July, 2 = August, 3 = September. (Error bars = +/- standard deviation.)**

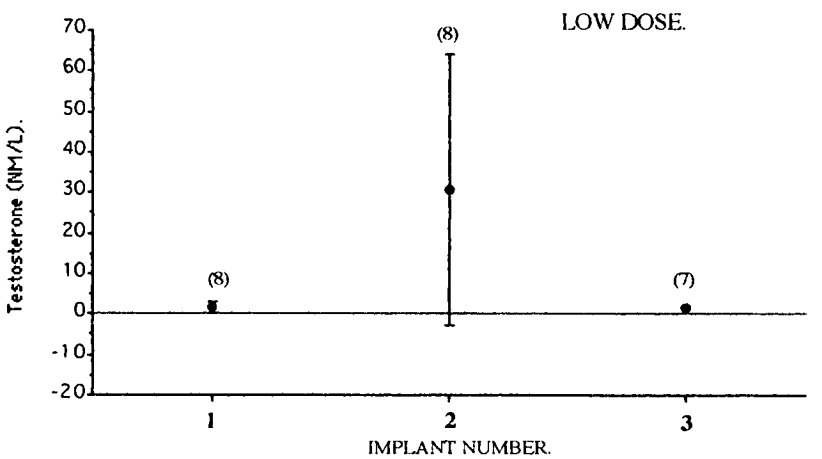


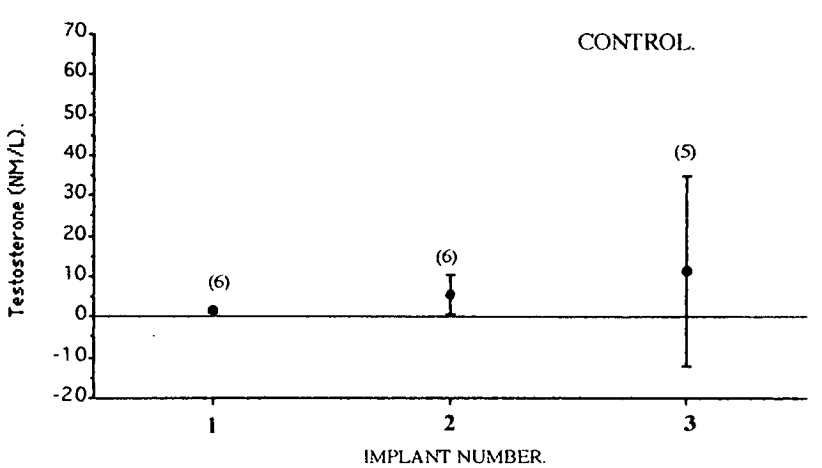




APPENDIX 26B: **DSF 1992 sustained release hormone implant experiment. Mean serum testosterone of *Latris lineata* for each experimental treatment, for each implant; 1 =July, 2 = August, 3 = September.(Error bars = +/- standard deviation.)**







APPENDIX 27: Kruskal-Wallis analysis of mean serum steroid levels of Latris lineata, for all treatments, at each implanting month.

IMPLANT MONTH	K statistic 17b-estradiol	K statistic Testosterone
JULY	0.291	2.687
AUGUST	2.778	1.647
SEPTEMBER	0.323	1.681

$K = \chi^2_{(2, 0.05)}$

(Ho: sig. diff. when $\chi^2_{(2,0.05)} \geq 5.991$)

APPENDIX 28: Mann-Whitney analysis of initial (0 hr) and +24 hr mean serum steroid levels of Latris lineata, for all treatments, following each monthly implant.

IMPLANT MONTH TREATMENT	MEAN RANK. 17b-estradiol Initial (0hr)	+24 hr	U-Stat.	MEAN RANK. Testosterone Initial (0hr)	+24 hr	U-Stat.
JULY						
High	7.88	3.75	27.0	7.50	2.00*	24.0*
Low	7.88	3.75	27.0	6.13	7.25	19.0
Control	5.64	6.63	16.5	3.67	8.25*	23.0*
AUGUST						
High	5.00	3.25	9.0	5.75	6.67	14.0
Low	6.00	3.00	14.0	7.13	5.25	21.0
Control	3.50	2.67	4.0	5.00	5.00	9.0
SEPTEMBER						
High	6.50	6.50	16.0	6.06	7.38	28.0
Low	6.86	4.50	20.0	6.36	5.38	16.5
Control	4.80	5.25	11.0	4.00	6.25	15.0

* = significantly different (P>0.05) by U-statistic.

Appendix 29: 1992 UTL sustained release hormone implant trial.
Mean oocyte diameters (μm) of hormone impalnted and control *Latris lineata* at each implanting month.

FISH NUMBER	TREATMENT	MEAN OOCYTE DIAMETER (um) / MONTH JULY	+/- S.E.	AUGUST	+/- S.E.	SEPTEMBER	+/- S.E.
300	HORMONE	196.8	11.3	399.3	25.0	131.0	0.0
*301	HORMONE	143.3	2.9	124.1	2.2	140.3	2.4
302	HORMONE	233.1	6.3	464.2	5.6	234.1	61.9
303	HORMONE	148.3	4.8	518.8	4.6	566.6	7.5
304	HORMONE	331.0	8.3	853.3	53.4	907.9	36.0
305	HORMONE	230.1	14.6	832.8	19.2	1030.7	59.5
*306	HORMONE	220.0	12.9	155.4	4.8	147.3	1.6
307	HORMONE	282.5	8.6	604.1	8.9	638.2	5.2
308	HORMONE	224.0	16.6	583.6	21.9	655.3	4.6
Mean		235.1	22.1	608.0	66.1	594.8	123.4
*313	CONTROL	N/A		N/A		N/A	
314	CONTROL	257.3	9.5	628.0	7.5	904.4	40.5
315	CONTROL	270.4	13.3	655.3	4.6	1249.2	13.7
Mean		263.9	6.6	641.6	13.7	1076.8	172.4

* = immature fish deleted from analyses.
N/A = no biopsy sample available

APPENDIX 30A: UTL experiment 1992.

Two way ANOVA of the effects of hormone implant treatment and implanting month on mean oocyte diameter of *Latis lineata* broodstock (P = 0.05).

Anova table for a 2-factor Analysis of Variance on Y_1: OOC.DIAM

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
IMPLANT (A)	2	1097606.637	548803.318	12.757	.0002
TREAT (B)	1	153654.36	153654.36	3.572	.0727
AB	2	210734.81	105367.405	2.449	.1106
Error	21	903440.977	43020.999		

There were no missing cells found.

The AB Incidence table on Y_1: OOC.DIAM

	TREAT:	IMPL.	CONTROL	Totals:
IMPLANT	A	7 235.097	2 263.854	9 241.487
	B	7 608.002	2 641.644	9 615.478
	C	7 594.835	2 1076.801	9 701.939
Totals:		21 479.311	6 660.766	27 519.635

APPENDIX 30B: UTL experiment 1992.

One way ANOVA of the effect of month of implanting, on mean oocyte diameter of hormone treated *Latis lineata* broodstock (P = 0.05).

One Factor ANOVA X_1: IMPLANT Y_1: OOC.DIAM

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	2	626833.211	313416.605	6.688
Within groups	18	843568.669	46864.926	p = .0067
Total	20	1470401.88		

Model II estimate of between component variance = 133275.84

One Factor ANOVA X_1: IMPLANT Y_1: OOC.DIAM

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
A	7	235.097	58.58	22.141
B	7	608.002	174.869	66.094
C	7	594.835	326.472	123.395

One Factor ANOVA X_1: IMPLANT Y_1: OOC.DIAM

Comparison: *	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
A vs. B	-372.905	243.135*	5.193*	3.223
A vs. C	-359.738	243.135*	4.832*	3.109
B vs. C	13.166	243.135	.006	.114

* Significant at 95%

APPENDIX 30C: **UTL experiment 1992.**

One way ANOVA of the effect of month of implanting, on mean oocyte diameter of control treatment *Latis lineata* broodstock (P = 0.05).

One Factor ANOVA X_1: IMPLANT Y_1: OOC.DIAM

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	2	661981.442	330990.721	16.585
Within groups	3	59872.308	19957.436	p = .0239
Total	5	721853.75		

Model II estimate of between component variance = 155516.642

One Factor ANOVA X_1: IMPLANT Y_1: OOC.DIAM

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
A	2	263.854	9.275	6.558
B	2	641.644	19.307	13.652
C	2	1076.801	243.749	172.357

One Factor ANOVA X_1: IMPLANT Y_1: OOC.DIAM

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
A vs. B	-377.791	449.587	3.576	2.674
A vs. C	-812.948	449.587*	16.557*	5.755
B vs. C	-435.157	449.587	4.744	3.08

* Significant at 95%

Appendix 31: 1992 UTL sustained release hormone implant trial. Serum levels of 17b-estradiol and testosterone of female *Latris lineata* sampled at implanting, and at 48 hr intervals following administration of each monthly implant.

			TIME AFTER IMPLANT (Hours)							
IMPLANT	TREATMENT	FISH	0		48		96		144	
MONTH		NUMBER	SEX STEROID E2= 17b-estradiol; T= Testosterone (NM/L).							
			E2	T	E2	T	E2	T	E2	T
JULY	Hormone	300	1.10	8.15	2.97	2.33	-	-	-	-
50ug LHRHa +	Hormone	302	5.89	13.39	3.14	2.74	-	-	-	-
200 ug 17a-MT	Hormone	303	3.70	4.22	-	-	0.29	2.08	-	-
	Hormone	304	15.49	11.23	-	-	12.38	3.31	-	-
	Hormone	305	8.86	5.56	-	-	10.56	3.92	-	-
	Hormone	307	N/A	4.44	-	-	-	-	8.26	2.95
	Hormone	308	10.35	3.72	-	-	-	-	4.29	2.59
Placebo	Control	314	5.32	3.53	-	-	-	-	6.46	4.72
	Control	315	4.15	4.20	-	-	-	-	10.78	5.65
AUGUST	Hormone	300	0.66	1.10	0.61	0.90	-	-	-	-
200ug LHRHa +	Hormone	302	0.62	1.20	0.68	3.10	-	-	-	-
200 ug 17a-MT	Hormone	303	0.80	N/A	-	-	1.40	1.60	-	-
	Hormone	304	17.71	4.10	-	-	21.12	1.40	-	-
	Hormone	305	26.83	3.10	-	-	25.09	6.30	-	-
	Hormone	307	4.55	1.30	-	-	-	-	12.24	42.40
	Hormone	308	5.78	1.90	-	-	-	-	11.45	10.90
Placebo	Control	314	15.69	51.20	-	-	-	-	4.44	5.20
	Control	315	4.13	N/A	-	-	-	-	3.36	1.20
SEPTEMBER	Hormone	300	0.53	26.90	1.55	N/A	-	-	-	-
200ug LHRHa +	Hormone	302	0.66	1.20	1.13	N/A	-	-	-	-
200 ug 17a-MT	Hormone	303	1.14	3.90	-	-	0.29	N/A	-	-
	Hormone	304	6.39	0.70	-	-	12.38	N/A	-	-
	Hormone	305	1.78	1.30	-	-	10.56	N/A	-	-
	Hormone	307	1.23	1.00	-	-	-	-	N/A	1.80
	Hormone	308	4.50	31.60	-	-	-	-	4.56	N/A
Placebo	Control	314	1.31	1.10	-	-	-	-	3.61	0.60
	Control	315	0.82	3.10	-	-	-	-	3.40	1.00

N/A = not available.

Appendix 32: 1992 UTL sustained release hormone implant trial. Two way ANOVA of mean sex steroid levels recorded at each month of impalnting for hormone treated, and control *Latris lineata*. a. Log_{10} 17β-estradiol. b. Log_{10} testosterone.

a.

Anova table for a 2-factor Analysis of Variance on Y_2: log(x) of E2

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
TREAT (A)	1	.008	.008	.033	.8586
IMPLANT (B)	2	1.504	.752	3.102	.0671
AB	2	.292	.146	.601	.5577
Error	20	4.848	.242		

3

There were no missing cells found.

The AB Incidence table on Y_2: log(x) of E2

	IMPLANT:	A	B	C	Totals:
TREAT	IMPL.	6 .756	7 .515	7 .2	20 .477
	CONTROL	2 .672	2 .906	2 .017	6 .532
Totals:		8 .735	9 .602	9 .159	26 .49

4

b.

Anova table for a 2-factor Analysis of Variance on Y_1: log(x) of TEST

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
TREAT (A)	1	.386	.386	2.063	.1672
IMPLANT (B)	2	.837	.418	2.239	.134
AB	2	1.882	.941	5.034	.0176
Error	19	3.551	.187		

1

There were no missing cells found.

The AB Incidence table on Y_1: log(x) of TEST

	IMPLANT:	A	B	C	Totals:
TREAT	IMPL.	7 .811	6 .27	7 .508	20 .543
	CONTROL	2 .586	1 1.709	2 .266	5 .683
Totals:		9 .761	7 .475	9 .455	25 .571

2

APPENDIX 33: 1992 UTL sustained release hormone implant trial.
Two way ANOVA for effect of hormone treatment and implanting month, on log_{10} mean serum sex steroid levels of hormone implanted and control *Latris lineata*, which ovulated batches of oocytes during this investigation.

a Log_{10} mean serum 17β-estradiol.

Anova table for a 2-factor Analysis of Variance on Y_1: log(x) of E2

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
DATE (A)	2	1.439	.72	12.501	.0004
TREAT. (B)	1	1.074	1.074	18.662	.0004
AB	2	.116	.058	1.004	.386
Error	18	1.036	.058		

There were no missing cells found.

The AB Incidence table on Y_1: log(x) of E2

	TREAT.:	IMPL.	CONTROL	Totals:
DATE	JULY	4 1.063	4 .797	8 .93
	AUG	4 1.35	4 .746	8 1.048
	SEPT	4 .679	4 .28	8 .48
Totals:		12 1.031	12 .608	24 .819

b Log_{10} mean serum testosterone.

Anova table for a 2-factor Analysis of Variance on Y_2: log(x) of TEST.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
DATE (A)	2	1.673	.837	6.066	.0117
TREAT. (B)	1	.064	.064	.464	.506
AB	2	.148	.074	.537	.5955
Error	15	2.069	.138		

There were no missing cells found.

The AB Incidence table on Y_2: log(x) of TEST.

	TREAT.:	IMPL.	CONTROL	Totals:
DATE	JULY	4 .727	4 .649	8 .688
	AUG	4 .512	3 .835	7 .651
	SEPT	2 -.02	4 .078	6 .045
Totals:		10 .492	11 .492	21 .492

APPENDIX 34: 1992 UTL sustained release hormone implant trial.
One way ANOVA for effect of hormone treatment on $\log_{10}$ mean serum 17β-estradiol levels at each implanting month, for hormone implanted and control *Latris lineata*, which ovulated batches of oocytes during this investigation.

a **JULY**

One Factor ANOVA X_1: TREAT. Y_1: log(x) of E2

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	.142	.142	6.85
Within groups	6	.125	.021	p = .0397
Total	7	.267		

Model II estimate of between component variance = .121

One Factor ANOVA X_1: TREAT. Y_1: log(x) of E2

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
IMPL. vs. CONTROL	.267	.249*	6.85*	2.617

* Significant at 95%

b **AUGUST**

One Factor ANOVA X_1: TREAT. Y_1: log(x) of E2

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	.73	.73	14.744
Within groups	6	.297	.049	p = .0086
Total	7	1.027		

Model II estimate of between component variance = .68

One Factor ANOVA X_1: TREAT. Y_1: log(x) of E2

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
IMPL. vs. CONTROL	.604	.385*	14.744*	3.84

* Significant at 95%

APPENDIX 34: 1992 UTL sustained release hormone implant trial.
(Cont.) One way ANOVA for effect of hormone treatment on $\log_{10}$ mean serum 17β-estradiol levels at each implanting month, for hormone implanted and control *Latris lineata*, which ovulated batches of oocytes during this investigation.

c SEPTEMBER

One Factor ANOVA X_1: TREAT. Y_1: log(x) of E2

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	.318	.318	3.105
Within groups	6	.615	.102	p = .1285
Total	7	.933		

Model II estimate of between component variance = .216

One Factor ANOVA X_1: TREAT. Y_1: log(x) of E2

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
IMPL. vs. CONTROL	.399	.554	3.105	1.762

Appendix 35: 1992 UTL sustained release hormone implant trial. Two way ANOVA comparing initial (0 hr) mean serum 17β-estradiol levels of *Latris lineata*, with levels 144 hr after each monthly implant.

a. July.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
TREAT (A)	1	.015	.015	.647	.48
TIME (B)	1	2.944E-5	2.944E-5	.001	.9741
AB	1	.096	.096	4.05	.1377
Error	3	.071	.024		

	TIME:	INIT.	SIX	Totals:
TREAT	IMPL.	1 1.015	2 .774	3 .855
	CONTROL	2 .672	2 .921	4 .797
Totals:		3 .787	4 .848	7 .822

b. August.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
TREAT (A)	1	.089	.089	1.485	.31
TIME (B)	1	.265	.265	4.445	.1256
AB	1	.023	.023	.388	.5775
Error	3	.179	.06		

	TIME:	INIT.	SIX	Totals:
TREAT	IMPL.	2 .372	1 .659	3 .468
	CONTROL	2 .017	2 .544	4 .28
Totals:		4 .195	3 .582	7 .361

c .September.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
TREAT (A)	1	.042	.042	.933	.3887
TIME (B)	1	.001	.001	.021	.8915
AB	1	.233	.233	5.156	.0857
Error	4	.181	.045		

	TIME:	INIT.	SIX	Totals:
TREAT	IMPL.	2 .71	2 1.073	4 .892
	CONTROL	2 .906	2 .586	4 .746
Totals:		4 .808	4 .83	8 .819

Appendix 36: 1992 UTL sustained release hormone implant trial. Two way ANOVA comparing initial (0 hr) mean serum testosterone levels of *Latris lineata*, with levels 144 hr after each monthly implant.

a. July.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
TREAT (A)	1	.037	.037	16.781	.0149
TIME (B)	1	.002	.002	.933	.3888
AB	1	.051	.051	23.01	.0087
Error	4	.009	.002		

	TIME:	INIT.	SIX	Totals:
TREAT	IMPL.	2 .609	2 .417	4 .513
	CONTROL	2 .586	2 .713	4 .649
Totals:		4 .597	4 .565	8 .581

b. August.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
TREAT (A)	1	.134	.134	1.027	.3854
TIME (B)	1	.012	.012	.095	.7783
AB	1	2.397	2.397	18.418	.0233
Error	3	.39	.13		

	TIME:	INIT.	SIX	Totals:
TREAT	IMPL.	2 .196	2 1.332	4 .764
	CONTROL	1 1.709	2 .398	3 .835
Totals:		3 .701	4 .865	7 .795

c .September.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
TREAT (A)	1	.289	.289	.693	.4663
TIME (B)	1	.304	.304	.73	.4558
AB	1	.006	.006	.013	.9158
Error	3	1.25	.417		

	TIME:	INIT.	SIX	Totals:
TREAT	IMPL.	2 .75	1 .255	3 .585
	CONTROL	2 .266	2 -.111	4 .078
Totals:		4 .508	3 .011	7 .295

APPENDIX 37: **1992 UTL sustained release hormone implant trial.**
One way ANOVA of the changes in $\log_{10}$ mean serum 17β -estradiol levels for hormone implanted *Latris lineata*, at 48 hr intervals following each monthly implant.

a. JULY

One Factor ANOVA X_1: TIME Y_1: log(x) of E2

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	.196	.065	.228
Within groups	9	2.577	.286	p = .8745
Total	12	2.773		

Model II estimate of between component variance = -.074

b. AUGUST

One Factor ANOVA X_1: TIME Y_1: log(x) of E2

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	2.128	.709	1.852
Within groups	10	3.83	.383	p = .2016
Total	13	5.958		

Model II estimate of between component variance = .109

c. SEPTEMBER

One Factor ANOVA X_1: TIME Y_1: log(x) of E2

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	.958	.319	2.702
Within groups	9	1.063	.118	p = .1082
Total	12	2.021		

Model II estimate of between component variance = .067

One Factor ANOVA X_1: TIME Y_1: log(x) of E2

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
A HR 0 vs. B HR+48	.079	.624	.027	.285
A HR 0 vs. C HR+96	-.598	.537*	2.118	2.521
A HR 0 vs. D HR+144	-.459	.831	.52	1.249
B HR+48 vs. C HR+96	-.677	.71	1.55	2.156
B HR+48 vs. D HR+144	-.538	.952	.544	1.277

* Significant at 95%

One Factor ANOVA X_1: TIME Y_1: log(x) of E2

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
C HR+96 vs. D HR+144	.139	.898	.041	.35

APPENDIX 38: 1992 UTL sustained release hormone implant trial.
One way ANOVA of the changes in $\log_{10}$ mean serum testosterone levels for hormone implanted *Latris lineata*, at 48 hr intervals following each monthly implant.

a. JULY

One Factor ANOVA X_1: TIME Y_2: log(x) of TEST.

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	.493	.164	4.917
Within groups	10	.334	.033	p = .0237
Total	13	.827		

Model II estimate of between component variance = .044

One Factor ANOVA X_1: TIME Y_2: log(x) of TEST.

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
A HR 0 vs. B HR+48	.409	.327*	2.589	2.787
A HR 0 vs. C HR+96	.334	.281*	2.337	2.648
A HR 0 vs. D HR+144	.393	.327*	2.401	2.684
B HR+48 vs. C HR+96	-.075	.372	.066	.447
B HR+48 vs. D HR+144	-.015	.407	.002	.083

* Significant at 95%

One Factor ANOVA X_1: TIME Y_2: log(x) of TEST.

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
C HR+96 vs. D HR+144	.059	.372	.042	.356

b. AUGUST

One Factor ANOVA X_1: TIME Y_2: log(x) of TEST.

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	1.869	.623	6.525
Within groups	9	.859	.095	p = .0123
Total	12	2.728		

Model II estimate of between component variance = .176

One Factor ANOVA X_1: TIME Y_2: log(x) of TEST.

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
A HR 0 vs. B HR+48	.047	.571	.011	.185
A HR 0 vs. C HR+96	-.114	.494	.09	.52
A HR 0 vs. D HR+144	-1.063	.571*	5.915*	4.213
B HR+48 vs. C HR+96	-.16	.638	.108	.569
B HR+48 vs. D HR+144	-1.11	.699*	4.298*	3.591

* Significant at 95%

One Factor ANOVA X_1: TIME Y_2: log(x) of TEST.

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
C HR+96 vs. D HR+144	-.949	.638*	3.775	3.365

* Significant at 95%

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