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Reproductive biology and larval morphology of the marine plotosid *Cnidoglanis macrocephalus* (Teleostei) in a seasonally closed Australian estuary

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Abstract

Monthly trends shown by gonadosomatic indices, the prevalence of the different gonadal stages, and the size distribution of the oocytes, indicate that the large marine and commercially important plotosid *Cnidoglanis macrocephalus* spawns in Wilson Inlet between October and January. The conclusion that spawning occurs within this seasonally closed estuary was confirmed by the presence of males in large nests and by the capture of newly-hatched, yolk sac larvae from one of those nests. The fact that *C. macrocephalus*, which is also widely distributed in coastal marine waters throughout much of southern Australia, can spawn within Wilson Inlet would be of particular value to this species in those periods when closure of the estuary would preclude a seawards spawning migration. Sexual maturity is size dependent, with spawning rarely occurring before fish have reached a total length of 425 mm. Sexual maturity was attained by a few fish at the end of their second year, by several at the end of their third year and by most, if not all fish, at the end of their fourth year. Comparisons with data for the more northern and permanently open Swan Estuary indicate that *C. macrocephalus* also spawns within that system and that the spawning time of this species is related to water temperature. The adult male guards the larvae under its pelvic fins in burrows. The larvae increased in total length from 29 mm just after hatching to 43 mm in the 17–18 days after capture, during which time their yolk sac was resorbed. Details are given of the morphology, morphometrics, meristics and pigmentation of larval *C. macrocephalus*. In comparison with the larvae of three other plotosid genera, the larva of *C. macrocephalus* is far larger in size and more developed at hatching and takes a shorter time to transform into a juvenile.

Introduction

The Plotosidae comprises the eel-tailed catfishes, which are represented in the marine, estuarine and fresh waters of the Indian Ocean and western tropical Pacific by about eight genera and 30 species (Hoese & Hanley, 1989). One member of this family, *Cnidoglanis macrocephalus*, which is found in inshore marine and estuarine waters throughout much of southern Australia southwards of latitutude 28°S (Kowarsky, 1976), makes an important contribution to the commercial fishery that is based in the estuaries of south-western Australia (Lenanton & Potter, 1987). Previous work on the age structure, growth rate and reproductive biology of the population of

C. macrocephalus in the Swan Estuary, on the lower west coast of Australia, provided circumstantial evidence that at maturity this species emigrates out of that estuary to marine spawning areas (Nel *et al.*, 1985). However, it is still not known whether *C. macrocephalus* is capable of breeding in estuaries and there is no information on the reproductive behaviour and larval morphology of this species.

During recent years, nearly half of the total commercial catch of *C. macrocephalus* in Western Australia has been obtained from Wilson Inlet (Anon., 1991). This estuary, which is located on the southern coast of Australia, becomes seasonally closed off from the sea through the formation of a sand bar at its mouth (Hodgkin & Clark, 1988). Since this closure usually occurs in the summer, at the time when *C. macrocephalus* breeds in Western Australia (Thomson, 1957; Nel *et al.*, 1985), any spawning migration out of the estuary would be prevented at those times. In this context, it is worth noting that the number of teleosts capable of breeding in the estuaries of south-western Australia is relatively high and that this has been assumed to reflect adaptations to the periods of landlocking to which these estuaries have been subjected during recent times (Potter *et al.*, 1986, 1990).

The present study was undertaken to ascertain whether monthly changes in indices of gonadal development provide evidence that the marine plotosid *C. macrocephalus* has become adapted to breeding within the seasonally closed Wilson Inlet. This approach has been complemented by attempts to ascertain whether this species constructs nests in the estuary, as is the case with the plotosid *Tandanus tandanus* in its riverine spawning areas (Lake, 1967a; Davis, 1977; Merrick & Midgley, 1981), and to determine where their newly-hatched larvae are found. The data and material collected during this study have also been used to examine the degree to which sexual maturity in *C. macrocephalus* is related to the size and/or age of the fish and to describe the morphology and growth of the larvae. The results obtained for *C. macrocephalus* in the seasonally closed Wilson Inlet are compared with those previously recorded for this species in the more northern and permanently open Swan Estuary (Nel *et al.*, 1985) in an attempt to elucidate which environmental variables are likely to influence gonadal development and spawning of this plotosid.

Materials and methods

Wilson Inlet consists of a large basin (48 km^2), which is fed by two main tributary rivers and opens to the Southern Ocean by a short and narrow entrance channel (Fig. 1; Hodgkin & Clark, 1988). Juvenile and adult *Cnidoglanis macrocephalus* were collected from Wilson Inlet between September 1987 and March 1989 using gill nets at eight sites (sites 1–8) distributed throughout the basin and at one site (site 9) in the saline region of the Hay River, one of the two main tributary rivers (Fig. 1). Sampling was carried out monthly at site 1 and in alternate months at all other sites. The gill nets comprised six panels (each 30 m long and 1.5 m high) with sequential stretched mesh sizes of 38, 51, 63, 76, 89 and 102 mm.

The total length (TL) and total weight of all male and female *C. macrocephalus* collected by gill nets in Wilson Inlet were recorded to the nearest 1 mm and 0.1 g, respectively. The gonad of each fish was removed and, on the basis of macroscopic appearance (see Laevastu, 1965), was assigned to one of the following gonadal stages: II = maturing virgin or recovering spent, III = developing, IV = maturing, V = mature, VI = spawning and VII = spent.

Each gonad was weighed to the nearest 0.1 g and the gonadosomatic index (GSI) calculated from the equation $W_g/W_f \times 100$, where W_g = wet weight of gonad and W_f = wet weight of fish, recorded in the same units. Each month, the gonads from three females collected from each of the six mesh sizes in the gill nets were fixed in 10% buffered formalin for 24 hours and then transferred to 70% ethanol. The diameters of oocytes, which fell on a straight line across the middle of the field of view, were measured to the nearest 0.1 mm using

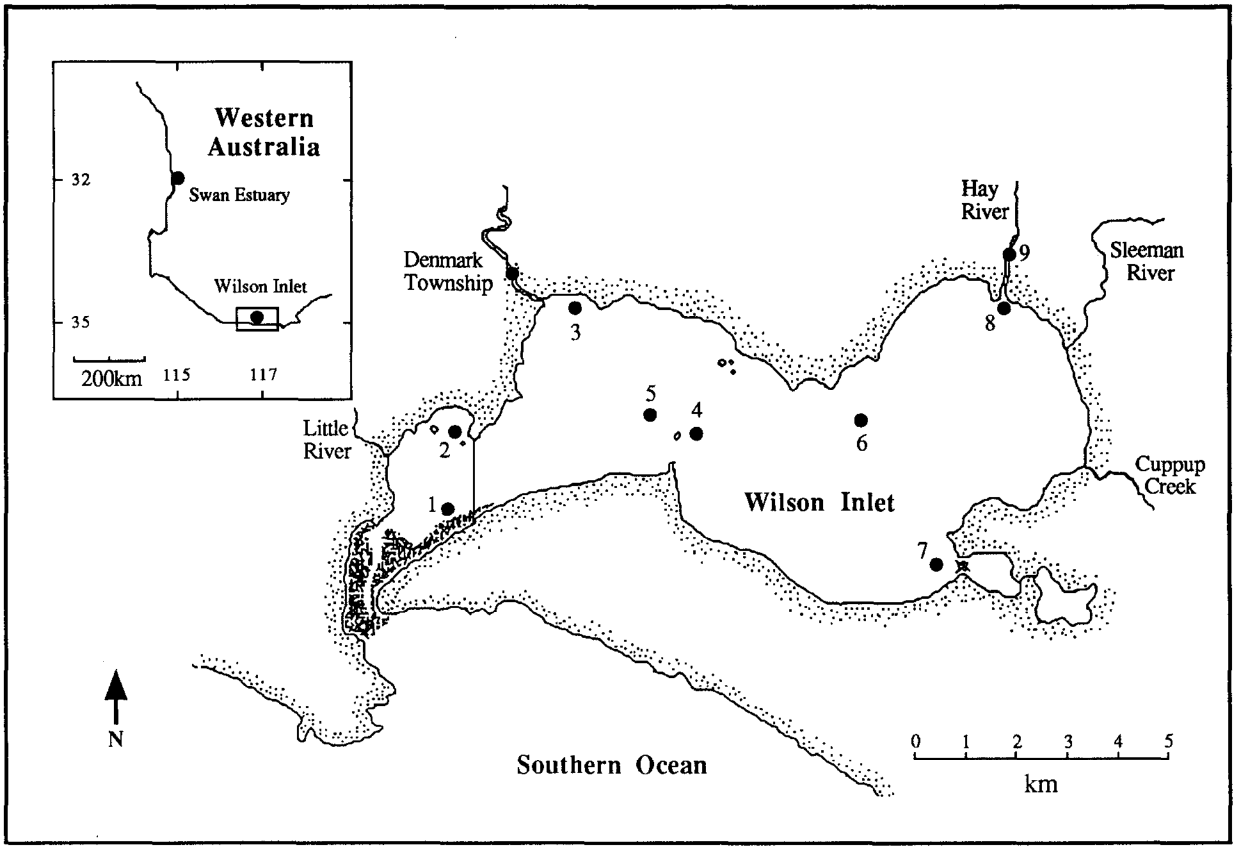


Fig. 1. Map showing the sites (1–9) from which *Cnidoglanis macrocephalus* were obtained in Wilson Inlet. Inset shows position of Wilson Inlet and the Swan Estuary in south-western Australia.

an eyepiece micrometer in a dissecting microscope.

Underwater observations were carried out in Wilson Inlet in December 1987 and 1988 and January 1988 and 1989 and in the Swan Estuary in November 1990 in an attempt to determine whether *C. macrocephalus* builds a nest.

A total of eight yolk sac larvae and eight early juveniles of *C. macrocephalus*, ranging between 27.0 and 38.0 mm standard length (SL), were used for measurements of body intervals and for describing the morphology, pigmentation and formation of fin rays. Terminology and body measurements follow Leis & Trnski (1989). The newly-hatched, yolk sac larvae (27.0–28.5 mm SL) were collected in December 1990 by dip net from a nest located at a depth of 1.5 m in the lower region of Wilson Inlet. Of the eight early juveniles, which came from the same area, five (34.0–36.5 mm SL) were caught using a conical, 500 μm mesh plankton net in shallow water in January 1989, while three (36.5–38.0 mm SL) were netted in a small 3 mm mesh beach seine in December 1990.

The standard length (SL), head length (HL), preanal length (PAL) and body depth (BD) of each of the 16 specimens, which had initially been fixed in 10% formalin and then preserved in 70% ethanol, were measured to the nearest 0.1 mm using an eyepiece micrometer in a dissecting microscope. Illustrations were made with the aid of a drawing tube fitted to the dissecting microscope. Body intervals are expressed as a percentage of SL. Pigment refers to the presence of melanin.

Four yolk-sac larvae and two early juveniles were cleared and double-stained (alcian blue for cartilage and alizarin red for bone), using the method described by Potthoff (1984), in order to facilitate the counting of fin rays and vertebrae and to determine the sequence of bone ossification. The term 'ossified' refers to structures that stained positively for bone.

Surface and bottom salinities and water temperatures in Wilson Inlet were recorded at the time of sampling. The data subsequently presented for these two environmental variables represents in each case the mean of the surface and bottom values. Day lengths on the 20th of each month in the regions of both Wilson Inlet and the Swan Estuary were obtained from the Perth Observatory. Salinity and temperature in the Swan Estuary represent values taken in the main sampling area during the two year study carried out on this species by Nel *et al.* (1985).

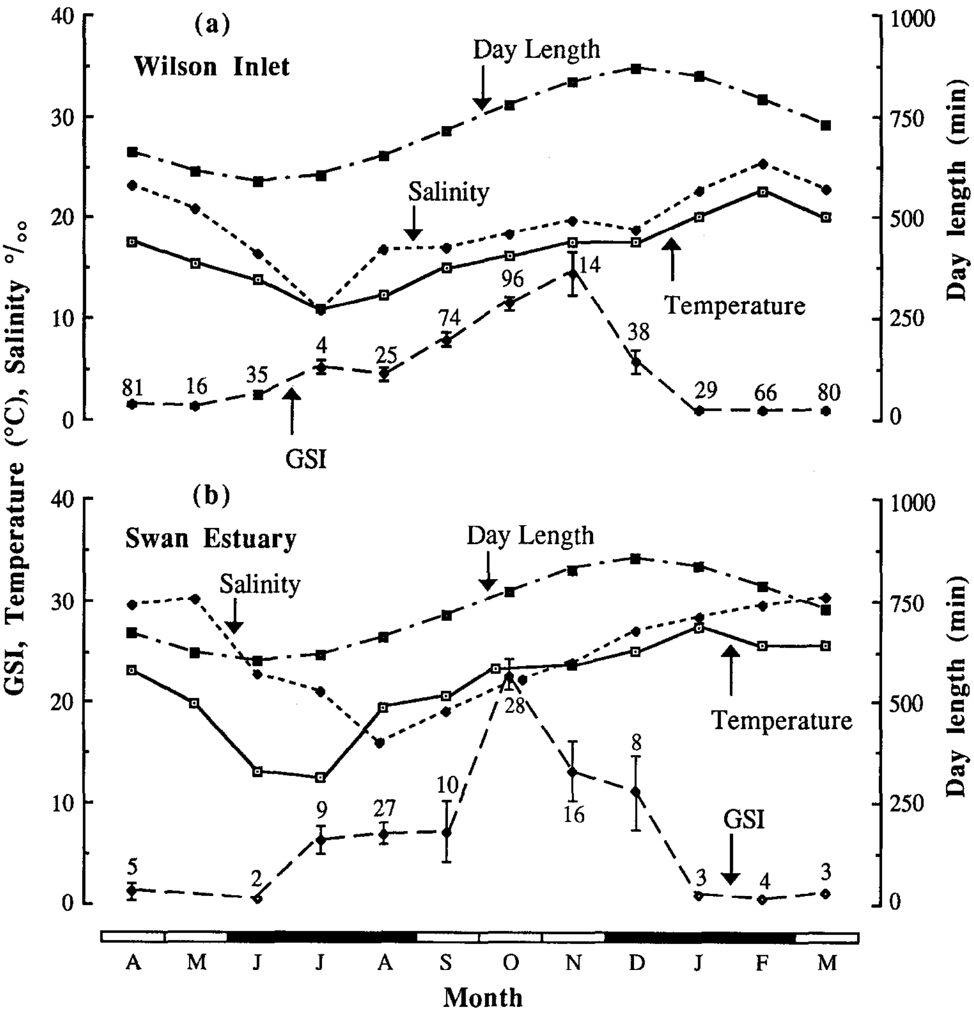


Fig. 2. Mean monthly values for salinity, water temperature and the Gonadosomatic index (GSI) of large *Cnidoglanis macrocephalus* in (a) Wilson Inlet (data from present study) and (b) the Swan Estuary (data from Nel *et al.*, 1985 and Potter *et al.*, unpublished). The day lengths in the regions of Wilson Inlet and the Swan Estuary on the 20th day of each month are also given. The GSIs for *C. macrocephalus* in Wilson Inlet and the Swan Estuary are for fish >425 and >385 mm TL, respectively, the lengths above which this species normally matures in the two systems. Means are based on data collected for Wilson Inlet between September 1987 and April 1989 and for the Swan Estuary between August 1982 and June 1984. On the horizontal axis, the open rectangles refer to the autumn and spring months and the black rectangles to the winter and spring months.

Results

Seasonal changes in environmental variables

Day length at Wilson Inlet gradually rose from its minimum of 588 min in June to its maximum of 870 min in December (Fig. 2a). Although day length followed the same trends in the locality of the Swan Estuary, the minimum (603 min) and maximum (854 min) were slightly higher and lower, respectively (Fig. 2b).

Mean monthly salinities in the basin of Wilson Inlet declined during the early autumn and winter to a minimum of 10.9‰ in July, before progressively rising to their peak of 25.2‰ in February (Fig. 2a). Although the trends in the Swan Estuary were similar, the mean monthly minimum (16.3‰) and maximum (30.3‰) salinities for the four sampling sites in this system were considerably higher (Fig. 2b).

Mean monthly water temperatures in the basin of Wilson Inlet increased from a low of 11.0 °C in July to a high of 22.5 °C in February (Fig. 2a). Although temperatures were generally higher in the Swan Estuary, they followed similar trends, rising from 12.5 °C in July to 27.4 °C in January (Fig. 2b).

Length at maturity

The females and males of *C. macrocephalus* collected in Wilson Inlet between December and February, when a preliminary examination of data

Table 1. Numbers of female and male *Cnidoglanis macrocephalus* > 425 mm with gonads at stages II to VII in samples collected from Wilson Inlet between September 1987 and April 1989.

Month	Gonadal stage					
	II	III	IV	V	VI	VII
FEMALES						
Jul		1	3			
Aug	3	2	19	18	1	
Sep	19	11	24	41	2	
Oct	14	10	27	6	2	2
Nov	1	1	1	8	4	1
Dec	10	4	2		3	11
Jan	17				7	5
Feb	45	5				16
Mar	49	15				16
Apr	60	18	2			1
May	9	7				
Jun	9	22	3	1		
MALES						
Jul						
Aug	1	3	1	2		
Sep	10		2			
Oct	3	7	5	3		
Nov		1	1			
Dec	1	2	7	7		
Jan	4	1		3	1	5
Feb	13	2		1		17
Mar	12	2	2			
Apr	18	6	6			
May			1			
Jun	3					

for the larger fish showed that gonadosomatic indices (GSIs) were falling from their peak in November and when many of the ovaries were at stage V (mature), VI (spawning) or VII (spent) (Table 1), were each grouped by length into 25 mm class intervals (Figs 3a, b). The contributions of the lengths of those fish possessing mature, spawning and spent ovaries have been highlighted on those length-frequency histograms (Figs 3a, b).

Ovaries at stages V, VI or VII were found in only three of the 142 female fish <425 mm, compared with 50 of the 136 females >425 mm, *i.e.* 94.3% of the mature, spawning or spent ovaries recorded in December to February came from female fish >425 mm (Fig. 3a). Similarly, testes at stages V, VI or VII were found in only one of the 116 fish <425 mm, compared with 34 of the 74 fish >425 mm (Fig. 3b), *i.e.* 97.1% of the fish with testes at stages V to VII came from fish >425 mm.

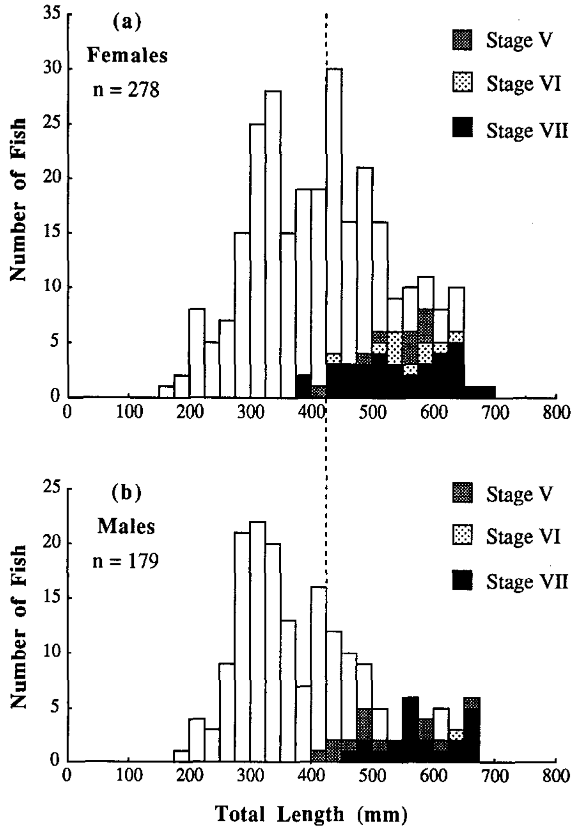


Fig. 3. Length-frequency histograms for all (a) female and (b) male *Cnidoglanis macrocephalus* caught during the summer (December to February) of 1987/88 and 1988/89. The contributions of the lengths of those fish which had gonads at stages V (mature), VI (spawning) and VII (spent) are shown.

Since very few fish attained sexual maturity at lengths <425 mm, the following description of the seasonal trends in GSIs and changes in gonadal stage and oocyte diameter have been based on data collected for fish above this length.

Gonadosomatic indices (GSIs)

The mean monthly GSIs of large female *C. macrocephalus* in both Wilson Inlet and the Swan Estuary were at their minimum (<2.5) between April and June (Figs 2a, b). In both systems, the mean GSIs gradually increased over the ensuing months to reach peaks of 14.5 in November in Wilson Inlet and of 21.8 in October in the Swan Estuary. The mean GSIs then declined rapidly in both systems and by January had fallen below 1.0. The highest individual GSI for a female in Wilson Inlet was the 31.8 recorded for a fish in November.

The paired testes of *C. macrocephalus* are very small, a point illustrated by the fact that the GSI for males with stage V (mature) gonads never exceeded 0.5. Despite this relatively small size, and the capture of only one male with a stage VI (spawning) testis, the GSIs showed similar seasonal trends to those of females (data not presented).

Gonadal stages

Stage III ovaries were found in females >425 mm in most months of the year (Table 1). Between late winter and late summer, the ovaries of maturing females became progressively more developed and then finally spent. Thus, stage IV ovaries were found mostly between August and October, stage V ovaries almost exclusively between August and November and stage VI ovaries predominantly between November and January. Stage VII (spent) ovaries were found mainly in fish caught between December and March (Table 1). Since the number of stage II ovaries (maturing virgin or recovering spent ovaries) increased progressively from only one in November to 45 in February and 60 in April, *i.e.* throughout the spawning and immediately post-spawning months, most of these ovaries are assumed to have been recovering spent and to have thus recently shed their eggs.

Males with stage V gonads were caught predominantly in December and January. The single male with a stage VI testis was caught in January. Males with spent testes were caught only in January and February. The prevalence of recovering spent testes increased from December to April.

Oocyte diameters

The oocytes in ovaries of females caught in December 1987 fell into two size groups, the first

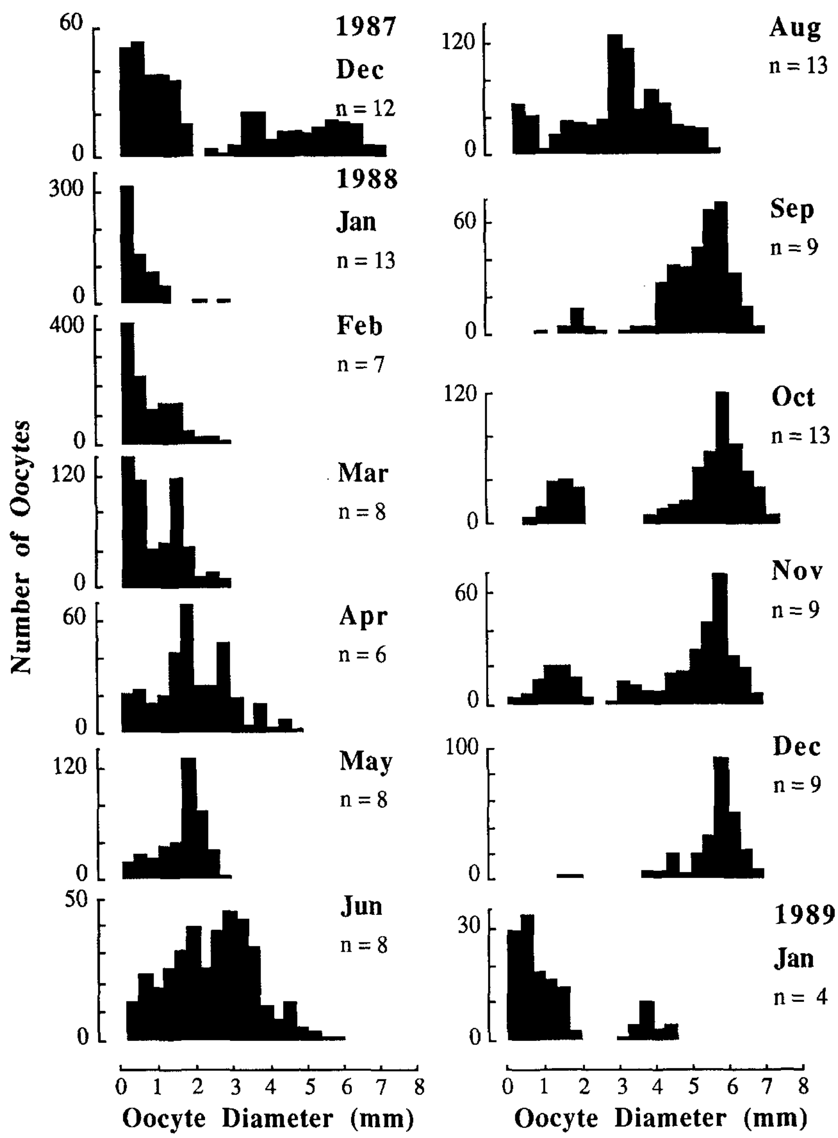


Fig. 4. Distribution of the diameters of oocytes in ovaries of large *Cnidoglanis macrocephalus* (≥ 425 mm TL) collected from Wilson Inlet between December 1987 and January 1989. n = number of fish sampled.

with diameters ranging up to 1.6 mm and the second with diameters lying predominantly between 3 and 7 mm (Fig. 4). By January 1988, the group of larger oocytes had essentially disappeared, and remained absent in the following two months. The upper limit in oocyte diameters rose from 2.7 mm in March 1988 to 7.3 mm in October. By this time, the oocytes again constituted two groups. A discrete group of larger oocytes was also present in October to December 1988, but this group had become greatly depleted one month later in January 1989. The distribution of oocyte diameters in January 1989 was similar to that in January 1988, except that there were several larger oocytes still present, but even then these were at the lower end of the size range of that group (Fig. 4). The largest oocyte diameter was the 7.3 mm recorded in October 1988.

Spawning period and date of birth

Since spent (stage VII) ovaries were first recorded in female fish in October and mature (stage VI) ovaries were not found after January (Table 1), *C. macrocephalus* presumably spawns in Wilson Inlet between these months. The spawning period in this estuary is thus only slightly longer than the one of October to December estimated by Thomson (1957) for *C. macrocephalus* in south-western Australian waters in general. However, it is worth noting that, since no large eggs, *i.e.* >5 mm in diameter, were left in the ovaries of the subsamples used for oocyte measurements in January of both 1988 and 1989 (Fig. 4), spawning in Wilson Inlet probably does not in general extend beyond December. Since the prevalence of spent (stage VII) and recovering spent (stage II) ovaries exceeded that of spawning ovaries for the first time in December (Table 1), spawning presumably peaked in late November and early December. Such a view is consistent with the marked fall that occurred in the GSIs of females between November and December (Fig. 2a). For the above reasons, *C. macrocephalus* has been assigned a birth date of 1 December in Wilson Inlet.

Age at maturity

No female or male fish caught between December and February with otoliths having one annulus possessed gonads at stages V, VI or VII (Fig. 5). Moreover, only one of the 58 females and two of the 56 males with otoliths possessing two annuli in this period had gonads at these advanced stages.

In contrast, the gonads of 28.7% of females and 29.4% of males with otoliths having three or more annuli between December and February were at stages V, VI or VII (Fig. 5). However, the prevalence of gonads at these stages is far less in

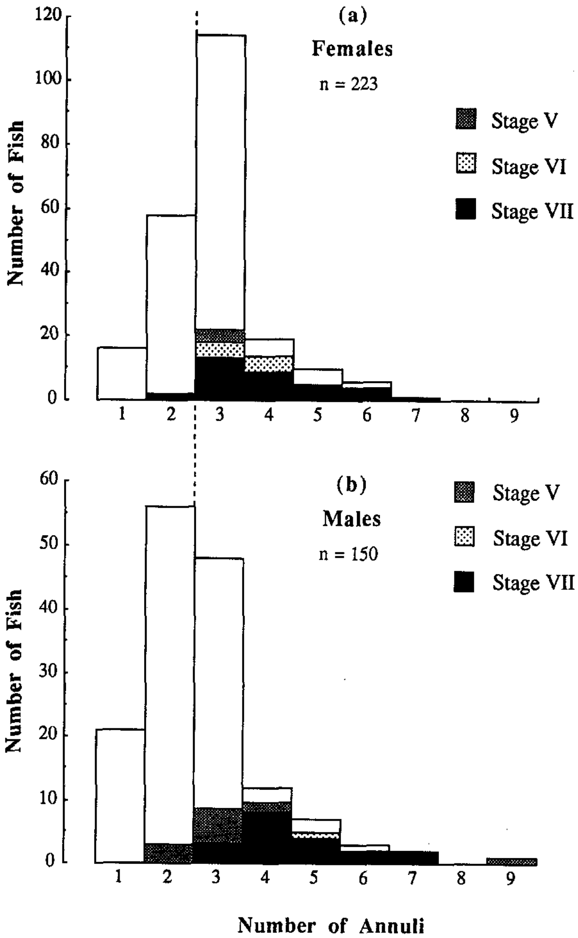


Fig. 5. The numbers of (a) female and (b) male *Cnidoglanis macrocephalus* possessing 1 to 9 otolith annuli during the summer (December to February) of 1987/88 and 1988/89. The contributions of the lengths of those fish with gonads at stages V (mature), VI (spawning) and VII (spent) are shown.

fish with otoliths having three annuli (22.7% for females and 16.3% for males) than with those having four or more annuli (52.1% for females and 57.5% for males). Furthermore, the mean total lengths of fish with otoliths having three annuli were significantly greater ($p<0.001$) in the case of those fish with gonads at stages V, VI or VII (males = 507 mm, females = 504 mm) than with those possessing gonads at stages II to IV (males = 446 mm, females = 461 mm).

Since the outer annulus of *C. macrocephalus* becomes detectable in the spring (Nel *et al.*, 1985; Laurenson *et al.*, in prep.), and the birth date of this species in Wilson Inlet (*i.e.* time of peak spawning) is 1 December, the fish with two, three, four annuli etc. in December to February correspond to those which on average would just have completed their second, third and fourth years of life, respectively.

Observations on reproductive behaviour

Approximately seven large and similar excavated holes were found in an area of approximately 1600 m^2 in the lower part of the basin of Wilson Inlet. Three of these excavations were each occupied by a single large male of *C. macrocephalus*. The burrows, whose substrate consisted of a mixture of shell debris and mud, generally extended under rocks. On 12 December 1989, an investigation was made of one of the burrows, which was located in 1.5 m of water. Although no larvae were observed in this burrow, the adult male remained in the burrow despite being prodded with a stick. Large numbers of the gastropod *Nassarius burchardi* were present in the burrow.

When the burrow was re-examined on the 13 December, the male fish was still present, but on this occasion there were approximately 100 *C. macrocephalus* yolk sac larvae resting at the base of its pelvic fins. The adult fish, which was removed from the burrow, measured 623 mm TL and weighed 1629 g. No female fish were observed in or around the burrow during the two days that the burrow was under observation.

The larvae were scooped into a plankton net, transferred to a holding tank and taken to the laboratory. The larvae swam when disturbed, but rapidly sank to the bottom when swimming ceased.

Larval growth and behaviour

The mean total length and weight of six larvae recorded in the field on the day of capture (13 December) was 29.3 mm and 0.25 g. The means ± 1 SEM for the length and depth of the yolk sacs of these larvae were 6.7 ± 0.1 mm and 5.3 ± 0.2 mm, respectively. The six larvae that survived transport to the laboratory were subsequently held in an aquarium for 24 days at 18 to 20 °C, *i.e.* at a water temperature similar to that of Wilson Inlet in December and January (Fig. 2). The larvae grew at a relatively uniform rate for most of the 17 days they possessed a yolk sac, increasing from 29 mm TL on the second day after capture to 43 mm TL, 14 days later, by which time the yolk sac of all larvae had been resorbed (Fig. 6). No further growth occurred during the following period despite attempts to forage for food on the substrate.

The pigmentation of larvae changed according to the colours present in the substrate, *i.e.* dark

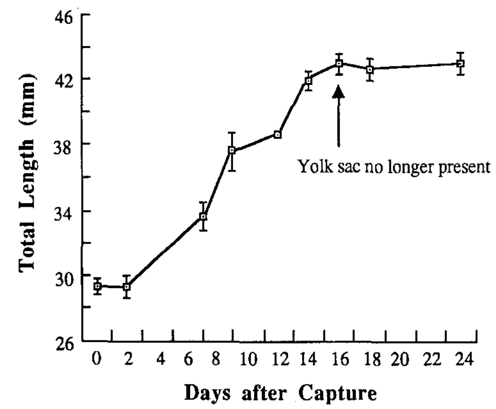


Fig. 6. Mean total length ± 1 SEM of larval *Cnidoglanis macrocephalus* collected from Wilson Inlet on 13 December 1989 and subsequently held for 24 days in aquaria maintained at 18–20 °C, which is similar to the water temperature in Wilson Inlet during that period. Larvae were taken from a burrow occupied by a guarding male fish of 623 mm.

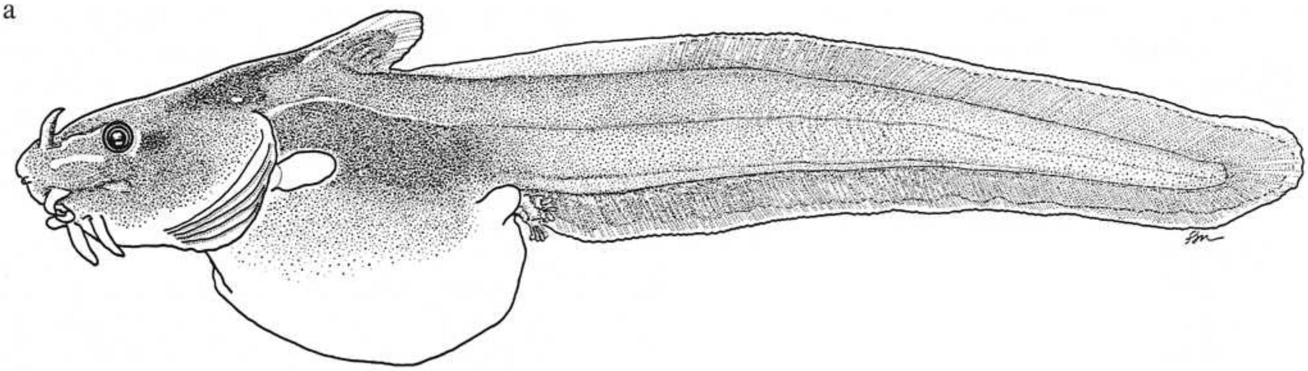
on dark substrates and light on light substrates. Although larvae attempted to burrow into the sand substrate by pointing their head downwards and swimming strongly, they were not successful in these attempts. The larvae sheltered in the gastropod shells and detached macrophytes that had been placed in the aquarium.

Morphology of larvae

Cnidoglanis macrocephalus larvae are characterised by an elongate body (BD = 14.5–18.2% SL), a head which is small in newly-hatched larvae (HL = 16.5–20.5% SL) and becomes moderate in early juveniles (HL = 20.7–24.8% SL) and a gut which remains of moderate length throughout development (PAL = 38.2–44.6 SL) (Figs 7a, b).

Larvae hatch between 27.0 and 28.5 mm SL with a functional mouth bearing a series of conical-like teeth in both jaws (non-ossified), all four pairs of mouth barbels, pigmented eyes, a large yolk sac and a dendritic organ posterior to the anus. At hatching, they also possess the buds of the pectoral and pelvic fins and developing first dorsal fin and the confluent second dorsal, caudal and anal fins (Figs 7a, b). All elements (non-ossified) were visible in the first dorsal (I, 3–5), anal (101–109) and caudal (7 + 2 = 9) fins in cleared and stained yolk sac larvae, whereas only the posterior 66–67 rays, of a total of 115–116 rays, were visible in the second dorsal fin (= dorsal procurrent caudal fin). All rays of the confluent second dorsal, caudal and anal fins (225–234), as well as the elements in each pectoral (I, 8–9) and pelvic (10) fin, are completely formed in early juveniles, *i.e.* > 34.0 mm SL. The eyes are laterally positioned in yolk sac larvae but migrate to a more dorsal position before settlement. The conical-like teeth in both jaws, the large spine of

a



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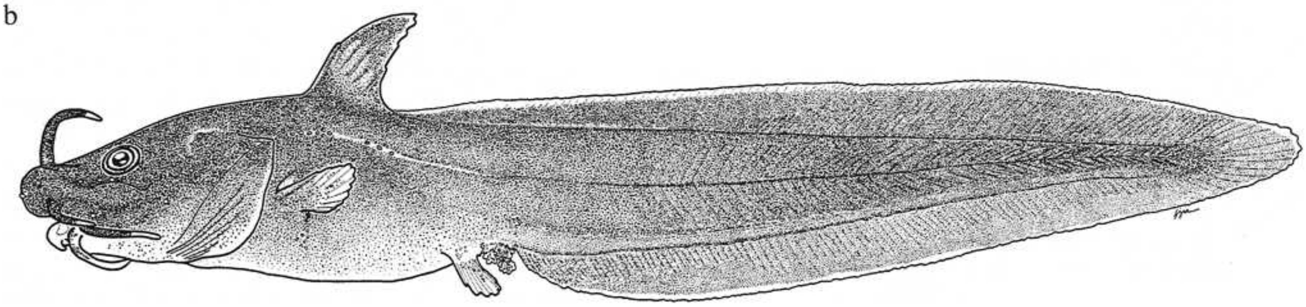

Fig. 7. a. 28.5 mm SL yolk sac larva of *Cnidoglanis macrocephalus*, collected in December 1989 from a nest in the lower region of Wilson Inlet. b. 35.5 mm early juvenile of *Cnidoglanis macrocephalus* collected in January 1989 from the surface waters of the lower region of Wilson Inlet.

the first dorsal fin and some of the anterior vertebrae were the only ossified structures that were visible in cleared and stained early juveniles.

Vertebral elements (non-ossified) are visible in cleared and stained newly-hatched larvae and start to ossify sequentially from the anterior to posterior regions by 34 mm. There are 77–78 vertebrae (12 preanal + 65–66 postanal).

Pigmentation

At hatching, the larvae have an overall pale yellowish coloration, with melanophores distributed over the whole body, except on the ventral surfaces of the head and yolk sac, the maxillary and mental barbels and the pectoral and pelvic fin buds. Early juveniles are more heavily pigmented, having dense pigment over the whole body surface, except on the ventral surfaces of the head and gut (Figs 7a, b).

Discussion

Spawning location

The females of *C. macrocephalus* caught in Wilson Inlet possessed ovaries ranging from the virgin stage (I), as in the small members of the 0 + age class caught in a separate study using seine nets, through to mature (V), spawning (VI) and spent (VII) stages, as in the larger fish caught during summer. The presence of a complete sequence of ovarian stages in these samples suggests that *C. macrocephalus* are adapted to spawn within this seasonally closed estuary. Such a conclusion is corroborated by the capture of newly-hatched, yolk sac larvae within the estuary. The capture of only one male with a stage VI (spawning) testis and the absence of spent males until late in the spawning season presumably reflects the fact that the brooding behaviour of males would make them inaccessible to capture by gill nets.

Since commercial catches in the Swan Estuary declined markedly just prior to and during the estimated spawning season of this species (late spring/early summer), Nel *et al.* (1985) proposed that the sexually maturing members of this population moved out of the estuary to breeding areas in nearby marine waters. However, since burrows characteristic of those described above for *C. macrocephalus* in Wilson Inlet were also observed in the Swan Estuary, at least some *C. macrocephalus* apparently spawn within the Swan Estuary.

Size and age at sexual maturity

This study has demonstrated that, for the population of *C. macrocephalus* in Wilson Inlet, the attainment of sexual maturity is size dependent, with few fish reaching maturity before their total length has reached 425 mm. These results are similar to those obtained by Nel *et al.* (1985) for *C. macrocephalus* in the Swan Estuary, where it was estimated that males and females reached sexual maturity at total lengths >405 mm and >385 mm, respectively. The gonads of the few $\geq 4+$ fish from Wilson Inlet in December to February that were not at stages V, VI or VII (Fig. 5), were all at stage II and thus likely to have been recovering spent, having shed their eggs early in the spawning season.

The present study showed that, in Wilson Inlet, maturity was attained by only a very few *C. macrocephalus* at the end of their second year, by several at the end of their third year (if they had attained a length of at least 425 mm) and by at least most fish at the end of their fourth and subsequent years of life. In contrast, in the Swan Estuary, maturity was reached by 22.5% of males and 14.0% of females at the end of their second year of life and by at least the majority of fish at the end of their third year of life (Nel *et al.*, 1985). The marked tendency for sexual maturity to be attained earlier in life by *C. macrocephalus* in the Swan Estuary is presumably related at least in part to the presence of a faster growth rate in that system. For example, at the end of the third year of life, the mean total lengths ± 1 SEM of the females of this plotosid were 436.3 ± 21.8 mm in the Swan Estuary, compared with 394.8 ± 8.2 mm in Wilson Inlet (Laurenson *et al.*, in prep.).

Spawning time and physico-chemical variables

Although the mean monthly GSIs for female *C. macrocephalus* exhibited well defined peaks in both Wilson Inlet and the Swan Estuary, these were attained one month later in the former system, *i.e.* in November rather than October. This implies that spawning peaks one month later in Wilson Inlet than in the Swan Estuary. Evidence from the different indices of gonadal development presented earlier indicates that, in Wilson Inlet, the spawning season of *C. macrocephalus* extends from October to January and peaks in late November/early December. If it is assumed that spawning in the Swan Estuary peaks soon after the mean GSIs start falling sharply from their maxima, as is the case with *C. macrocephalus* in Wilson Inlet, the spawning of *C. macrocephalus* in that more northern estuary would peak in late October/early November. A later spawning in Wilson Inlet would be consistent with the lower water temperatures found in that system. However, even though spawning takes place a month later in Wilson Inlet than in the Swan Estuary, the mean water temperature when spawning peaked in Wilson Inlet (*ca* 18 °C) had still not reached that at which spawning peaked one month earlier (*ca* 22 °C) in the Swan Estuary. The latter temperature is similar to the 24 °C which, in the case of the freshwater plotosid *Tandanus tandanus*, appeared to be the 'primary factor stimulating spawning' (Davis, 1977).

The environmental data shown in Fig. 2 indicate that *C. macrocephalus* exhibited peak spawning activity in Wilson Inlet when salinities were less than 20‰ and in the Swan Estuary when salinities were about *ca* 22‰. As this species also spawns in marine environments (35‰), breeding is not linked to the presence of full strength sea water.

Since day length in the regions of the two systems differs only slightly, it seems unlikely that this variable influences the spawning time. However, it is worth noting that both day length and water temperatures start rising in the late winter and early spring, and that this is the period when the gonads start to undergo rapid development.

Larval guarding, morphology and growth

Since the excavated burrows found in Wilson Inlet were usually located in fine sand and under large rocks, efforts to explore their interior generally resulted in the collapse of the surrounding walls. However, it was possible on three occasions to examine the inside of a burrow and in each of these cases they were found to contain a large adult male. Nests are also built by the freshwater plotosid *Tandanus tandanus*, these typically being circular and saucer-shaped depressions constructed in a substrate generally comprising at least some gravel (Lake, 1967a; Davis, 1977). From the single occasion when the inside of the burrow could be fully explored, it is apparent that the male remains in the burrow during at least the first day after the larvae are hatched and, from the absence of spent males in samples taken by gill net during the first weeks of the spawning period, they may do so until the yolk sac of these larvae has been resorbed. Moreover, the male apparently guards the larvae under its pelvic fins.

At hatching, the larvae of *Cnidoglanis macrocephalus* are more developed and larger (27.0–28.5 mm SL) than those of the other three species of the Plotosidae for which there is information on the morphology of their larvae. Thus, while the larva of each of the other three species, namely *Tandanus tandanus*, *Neosilurus ater* and *Plotosus lineatus*, also hatch with a large yolk sac, they lack for example fin elements, and measure only 5.7 to 7.4 mm (Lake, 1967b; Orr & Milward, 1984; Hosoya, 1988). The far greater size at which *C. macrocephalus* hatches is related to the larger diameter of its fully-developed eggs, *i.e. ca* 7.4 mm (Nel *et al.*, 1985; this study) compared with 3.1–3.4 mm in *T. tandanus* and *ca* 2.6 mm in *N. ater* (Lake, 1967b; Orr & Milward, 1984). In addition, larval *C. macrocephalus* transforms into a juvenile in a shorter time than these two species (*ca* 17–18 *vs* 24 and 28 days, respectively) and at a much larger size than those of the above two plotosids (*ca* 34 mm SL *vs* 15 and 25 mm TL, respectively).

Since larval *C. macrocephalus* grew at an average rate of *ca* 0.9 mm per day for 17 to 18 days, finally reaching a total length of *ca* 43 mm TL when the yolk of all fish had been resorbed, it attains within this period approximately a fifth of the expected length of the fish at the end of its first year of life (Laurenson *et al.*, in prep.). The fact that the above mean length of 43 mm attained by larvae in an aquarium was greater than the 35.5 mm of the early juvenile caught by plankton net in Wilson Inlet and shown in Fig. 7b, suggests that *C. macrocephalus* might transform into a juvenile at a smaller size in the estuary than under captivity. However, it should be recognised that, for the *C. macrocephalus* held in captivity, the measurements represented total rather than standard lengths and were made on live fish rather than on fish that had been preserved, a process that would presumably have led to some shrinkage. Moreover, some of the larvae in the aquarium did hatch at lengths less than 40 mm TL.

The advanced stage of development of larval *C. macrocephalus* at hatching, the guarding of its eggs and newly-hatched larvae within the nest by the adult male, and the relatively rapid transition from larva to juvenile would help maximize survival within the fluctuating conditions which characterise estuaries. Reproductive strategies involving the protection of eggs and/or larvae by the adults are also employed by some other teleosts which spawn in south-western Australian estuaries. For example, the males of *Apogon rueppellii* brood the eggs in its mouth until the larvae hatch (Chrystal *et al.*, 1985; Neira, 1992), and the males of syngnathids brood their eggs and newly-hatched young in ventral pouches (Breder & Rosen, 1966).

In summary, *C. macrocephalus* breeds within the seasonally closed Wilson Inlet and the males of this species guard the larvae underneath their pelvic fins in burrows which they excavate in the substratum of the estuary. The same situation probably pertains to at least some members of the population of *C. macrocephalus* found in the permanently open Swan Estuary. The attainment of sexual maturity in Wilson Inlet is size dependent, rarely occurring at total lengths less than 425 mm, and thus does not tend to occur until the fish is at the end of the third year of life. The larvae grow at a rate of *ca* 0.9 mm a day until they reach *ca* 43 mm, by which time they have all resorbed their yolk sacs.

The ability of *C. macrocephalus* to breed within Wilson Inlet parallels the situation described for another marine species, the flathead *Platycephalus speculator*, which likewise lives for several years in this system and attains lengths in excess of 600 mm (Hyndes *et al.*, 1992a, b). The fact that these longer-lived marine species can breed within estuaries presumably reflects adaptations to overcome the problems of emigrating to marine spawning areas from estuaries which are at times landlocked. This view is endorsed by the fact that the overall prevalence of estuarine spawning species in Wilson Inlet is relatively high compared with the situation in constantly and very well flushed northern hemisphere estuaries, such as that of the River Severn in the south-western region of the United Kingdom, where the fish fauna is dominated by the juveniles of species spawned at sea (Claridge *et al.*, 1986).

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