Seasonal Ecology, Condition and Reproductive Patterns of the Smooth Toadfish *Tetractenos glaber* (Freminville) in the Hawkesbury Estuarine System, Australia

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Seasonal variation in population structure, diet, lipid condition and reproduction of the smooth toadfish, *Tetractenos glaber*, were monitored in Berowra Creek and Cowan Creek, two estuarine arms of the Hawkesbury River, New South Wales, Australia. *Tetractenos glaber* is site-specific, feeding on benthic mussels and crustaceans. Reproduction occurs in April-July (winter), preceded by a buildup of somatic lipids in both sexes in February–April (Autumn). The liver appears to be a major source of lipid mobilisation, with total lipids varying among individuals from 20 to 90% of liver dry weight, and from 6 to 30% for muscle tissue. Recruitment occurred in November, and populations were composed of individuals up to at least 13 years of age. Sex ratios fluctuated seasonally, with a higher proportion of females sampled in winter, and more males in summer. The site-specificity, high abundance and benthic foraging behaviour of *T. glaber* suggest that it is largely estuarine-resident, and make it a potential bioindicator of the effects of degraded water quality on estuarine biota.

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INTRODUCTION

Estuaries provide organisms with many physiological challenges, including fluctuations of salinity, temperature, turbidity and dissolved oxygen, at a variety of temporal and spatial scales. Fishes are the major vertebrate taxon in estuaries and exhibit a wide range of life histories. Some are only seasonally resident, or pass briefly through as they migrate between fresh to marine waters (e.g., salmon, eels). Others spend most of their adult life in estuarine waters, taking advantage of high productivity and food levels, and many of these species are of commercial importance. Reduced water quality may affect community structure, populations and individual physiology of fishes. While lethal effects of point-sources of pollution on fishes have been demonstrated (e.g. Sakthivel and Gaikwad 1994), chronic, sublethal effects of degraded waterways may be of more widespread importance to estuarine communities. Species diversity of fish assemblages is often reduced in polluted waters, and population densities of some species may also be reduced (e.g., Lee et al. 1992). Lipid concentration is an important somatic measure of physiological condition in fishes, and may be influenced by seasonal changes in diet, reproduction or pollution level (Booth and Keast 1986; Fraser 1989; Suthers et al. 1992). A drop in the somatic condition (measured as lipid depletion) of individual fish in winter may interact with external stressors to cause increased mortality ("Winter Stress

Syndrome": Lemly 1996). Fraser (1989) suggested that the quantity of storage lipids, such as triacylglycerides (TAG), are related to ambient pollution levels for clupeid larvae, while Wang and Stickle (1988) found that TAG quantity was inversely related to exposure concentration to crude oil for juvenile blue crabs (*Callinectes sapidus*).

The present study investigates seasonal changes in the ecology and physiology of a common fish, the smooth toadfish *Tetractenos glaber*, in Berowra Creek and Cowan Creek estuaries, on the northern outskirts of Sydney, Australia. The overall goals of the study were to determine seasonal patterns in the life history of a common estuarine inhabitant, and to assess its use as a bioindicator of degraded estuarine health. Specifically, we asked

- is the species resident at sites within the Creeks?
- do population structure and sex ratio change seasonally, and when does recruitment occur?
- what are the main dietary items, and do quantity and quality of diet change seasonally?
- are there links between seasonal reproductive cycles and somatic condition?
- would *T. glaber* be a useful biomonitor of estuarine health?

MATERIALS AND METHODS

Study species

Tetractenous glaber (smooth toadfish) is a small tetraodontid fish inhabiting estuaries and coastal bays on the south-east coast of Australia (Kuiter 1994). It is found in aggregations in mud-flat shallows in estuaries, and may enter freshwater, but is not fished commercially due to the toxin it carries on its skin and in its internal organs. Few studies have focussed on tetraodontid fish ecology, despite their major contribution to fish biomass in estuaries and other habitats (e.g., Thresher 1984). Toadfishes are known to forage on a range of estuarine benthic organisms, including gastropods and oysters, and may significantly affect inter- and subtidal assemblages through predation (Connell and Anderson 1999).

Study sites

Fish were sampled in two major estuarine arms of the Hawkesbury River (33°30′S, 151°10′E), Cowan Creek and Berowra Creek, from October 1995 to March 1997, with sampling carried out at six different sites (4 in Berowra Creek, 2 in Cowan Creek, see Fig. 1) every 1–2 months. The Hawkesbury River extends over 200 km and flanks the Sydney Basin to the north and west. It supports significant recreational and commercial fisheries, particularly prawns, and is used for mariculture of oysters along the foreshores (Mercer 1984).

Berowra Creek is a sheltered waterway with a limited tidal flow, and urban development which have generated considerable sediment movement. Its tributaries are sites for sewage treatment plants (Fig. 1). Urban and farm runoff cause significant pollution and may be major factors affecting the quality of the water and sediment (unpublished data). Cowan Creek, part of the Ku-ring-gai Chase National Park, is located on the southern side of Broken Bay, about 5 km east of Berowra Creek (Fig. 1). The major habitats found within this creek include mangroves and sandflats with sparse seagrass areas. While similar in morphology, Cowan Creek is generally cleaner and more saline than Berowra Creek, due to its closer proximity to the ocean, reduced urban and agricultural

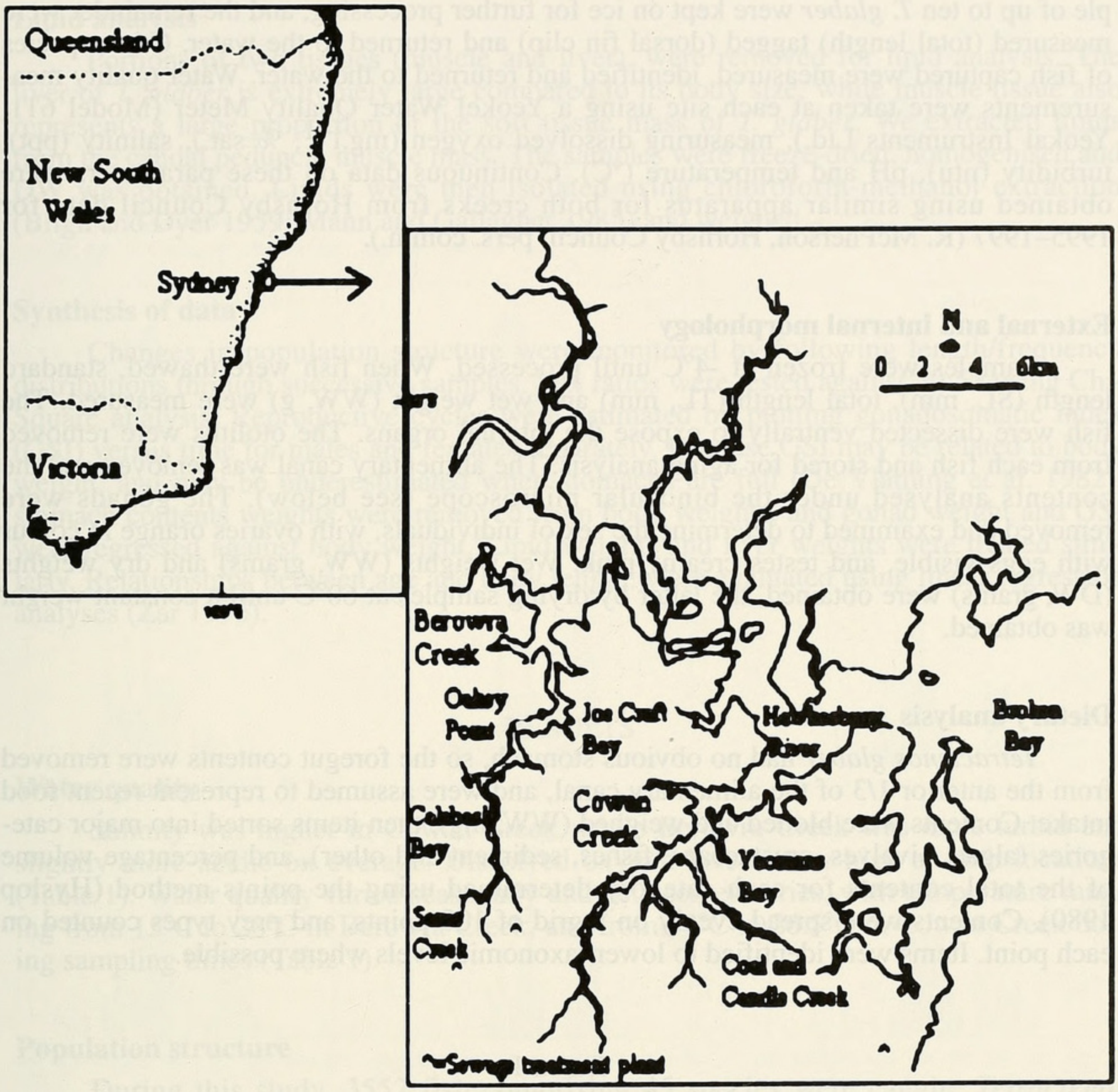

Figure 1. Lower Hawkesbury estuary, showing Berowra Creek (Oakey Poin, Joe Crafts Creek, Calabash Bay, Sams Creek) and Cowan Creek (Yeomans Bay, Coal and Candle Creek) study locations.

runoff, and lack of sewage treatment plant effluent (Hornsby Council, unpub. report). However, it receives considerable recreational boat traffic. Natural oysters are prolific along the rocky shores, compared to Berowra Creek, where rocky foreshores that once supported oysters, are now covered with green algae. Both creeks have sediment "hotspots" for contaminants such as DDD, and other derivatives of DDT along with high concentrations of nitrogen and phosphate nutrients (Birch et al. 1998).

Field sampling

Fish were taken using a 15.5 x 1.75 m beach-seine net with a mesh of 16 mm stretch. The method involved manually hauling the net across the bottom, covering a depth of up to 1.5 m and a width of about 8–10 m, encompassing an area of about 10–15 m^2. Sampling at all sites was carried out for 2 hours either side of the afternoon low tide (1400h to 1600h in winter, 1500h to 1700h in summer). From each haul, a random sample of up to ten *T. glaber* were kept on ice for further processing, and the remainder were measured (total length) tagged (dorsal fin clip) and returned to the water. Other species of fish captured were measured, identified and returned to the water. Water quality measurements were taken at each site using a Yeokel Water Quality Meter (Model 611, Yeokal Instruments Ltd.), measuring dissolved oxygen (mg.l^{-1}, % sat.), salinity (ppt), turbidity (ntu), pH and temperature (°C). Continuous data on these parameters were obtained using similar apparatus for both creeks from Hornsby Council data for 1995–1997 (R. McPherson, Hornsby Council, pers. comm.).

External and internal morphology

Samples were frozen at -4°C until processed. When fish were thawed, standard length (SL, mm), total length (TL, mm) and wet weight (WW, g) were measured. The fish were dissected ventrally to expose the internal organs. The otoliths were removed from each fish and stored for aging analysis. The alimentary canal was removed and the contents analysed under the binocular microscope (see below). The gonads were removed and examined to determine the sex of individuals, with ovaries orange in colour with eggs visible, and testes creamy-pink. Wet weights (WW, grams) and dry weights (DW, grams) were obtained, the latter by drying samples at 60°C until a constant weight was obtained.

Dietary analysis

Tetractenos glaber had no obvious stomach, so the foregut contents were removed from the anterior 1/3 of the alimentary canal, and were assumed to represent recent food intake. Contents were blotted and weighed (WW, mg), then items sorted into major categories (algae, bivalves, crustaceans, fishes, sediment and other), and percentage volume of the total contents for each category determined using the points method (Hyslop 1980). Contents were spread evenly on a grid of 100 points, and prey types counted on each point. Items were identified to lower taxonomic levels where possible.

TABLE 1.

Variation in water quality parameters in Berowra Creek and Cowan Creek, February 1996 to March 1997 [mean (se), n=16 sampling times for Berowra, 7 for Cowan]. Ranges refer to data obtained over the study period (McPherson, pers. comm.) using similar apparatus on a continuous logging basis at a site in each creek from 1995–1997.

		Berowra Creek	Cowan Creek
Temp	Mean (se):	21.1 (0.5)	21.2 (0.7)
(8C)	Range:	14.8–28.0	16.7–26.5
Salinity	Mean (se):	17.7 (1.3)	27.0 (1.8)
%o	Range:	16.1–19.5	24.9–30.0
pH		Mean (se):	7.7 (0.05) 8.0 (0.03)
Range:	7.6–8.0		7.9–8.1
Dissolved O_2	Mean (se):	7.7 (0.2)	7.6 (0.2)
(mg/l)	Range:	7.4–8.0	7.3–7.9
(% sat)	Mean (se):	94.3 (2.2)	102 (1.4)
Turbidity	Mean (se):	4.6 (1.1)	0.8 (0.1)
(ntu)	Range:	3.3–6.9	0–2.2

Lipid analysis

Portions of two tissues (muscle and liver), were removed for lipid analysis. The liver of *T. glaber* is extremely large compared to its body size, while muscle tissue also represents a large proportion of the soft tissue mass of *T. glaber*. We extracted lipids from the caudal peduncle muscle mass. The samples were freeze-dried, homogenised and DW was obtained. Lipids were then isolated using chloroform-methanol extraction (Bligh and Dyer 1959; Mann and Gallagher 1985) and weighed.

Synthesis of data

Changes in population structure were monitored by following length/frequency distributions through successive samples. Sex ratios were tested against 50:50 using Chi-Square analysis. Reproductive cycles were estimated by plotting gonadosomatic index (GSI) versus time for males and females separately. Because GSI may be related to body weight, and may be underestimated when stomachs are full (De Vlaming et al. 1982), stomach contents weights were removed from body weights, and gonad weight and GSI were regressed against body weight. Lipid weights and liver weights were treated similarly. Relationships between age and body length, were evaluated using linear regression analyses (Zar 1996).

RESULTS

Water quality

Salinity was higher in Cowan Creek, while Berowra Creek was more turbid and slightly more acidic on average. Dissolved oxygen levels were high at both locations (Table 1). Water quality varied seasonally and between estuaries, with temperature ranging from 15°C to 28°C in Berowra Creek, and from 17°C to 26.5°C in Cowan Creek during sampling times (Table 1).

Population structure

During this study, 3552 fish comprising 45 species were caught. *Tetractenos glaber* was represented at all sites and times, and comprised 43% of the total fish catch (n=1527 fish). However, catches of *T. glaber* at each of the sites varied temporally, with very low numbers caught at some sites on some occasions, so data were pooled for Berowra Creek sites and for Cowan Creek sites. Although differences among sites within locations were therefore not assessed, the use of multiple sites in each Creek gave increased spatial generality to results within estuaries. Few *T. glaber* were caught during the study from Cowan Creek, partly due to the difficulty of accessing habitat using seine nets, but perhaps as a result of higher salinity there. Video observations on seine net operation in both creeks confirmed that net avoidance was rare for this species (unpublished data).

Fish ranged from 32 to 160 mm TL, with new recruits (<40 mm TL) appearing in November 1995 at Berowra Creek sites (Fig. 2). This cohort could be traced until March 1996, when it merged with the size classes above 80 mm TL. However, the November 1996 pulse was much smaller. There was a linear relationship between age (as determined from otoliths) and body length (r^2= 0.89, n=8, p<0.05; Central Aging Service, pers. comm.), with the largest fish (160 mm TL) being 13 years of age, and the smallest (32 mm TL) being under one year old. Apart from recruitment pulses, the size-distribution of fish in Berowra Creek was similar throughout the study. Too few fish were caught in Cowan Creek, but ranges were similar to Berowra Creek samples.

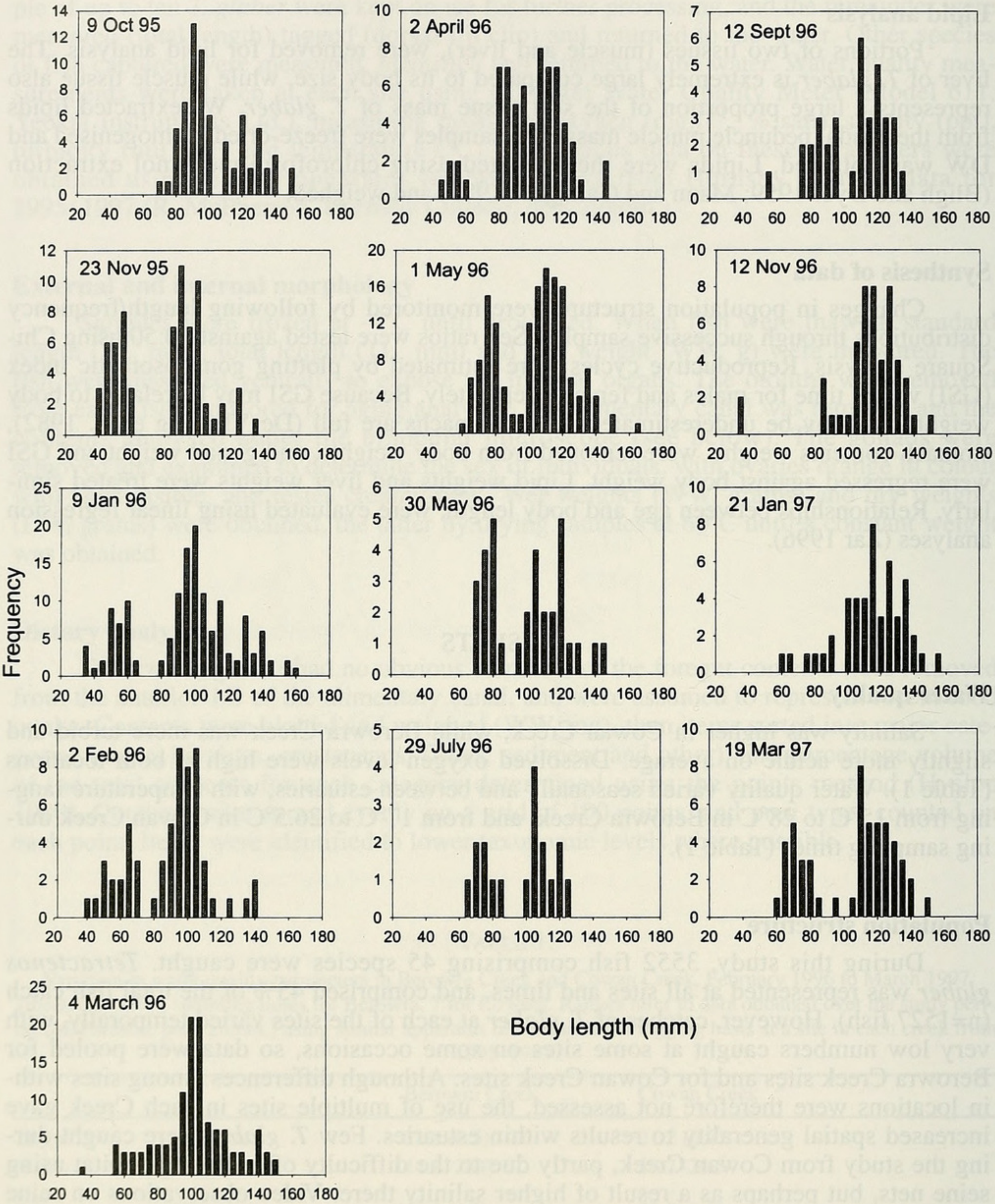

Figure 2. Length-frequency distributions for *T. glaber* at 4 sites (pooled) in Berowra Creek estuary, from October 1995 to March 1997. (Body length: total length, mm; length classes: 5 mm)

Movement of tagged fish

Tagging of fishes in Berowra Creek indicated a 10–11% recapture overall of tagged fish (Table 2). This rate of recapture was highly variable among dates, suggesting mortality or some degree of emigration from sites, although no tagged fish were recovered at adjacent sites during the study. In addition, snorkeling surveys conducted during the study suggested that this species rarely inhabited deeper adjacent waters.

TABLE 2.

Tagging and recaptures of *T. glaber* in Berowra Creek estuary. "Total % recap" refers to (a) the percentage of either fish tagged on the previous tagging date ("Last tag date"), the sum of the previous 2 dates ("Last 2 tags") or the sum of the previous 3 sampling dates ("Last 3 tags"), that were recaptured.

Date	# caught	# tag/release	# recap	Total % recaptured		
				Last tag date	Last 2 tags	Last 3 tags
2 April 96	42	34	–	–	–	–
1 May 96	70	51	1	2.9	–	–
30 May 96	15	13	5	9.8	5.8	–
12 Sep 96	21	21	0	0	0	0
12 Nov 96	31	19	5	23.8	13.9	5.7
21 Jan 97	37	32	7	36.8	17.5	12.7
19 Mar 97	19	9	1	3.1	2	1.3
Total	**233**	**181**	**19**			

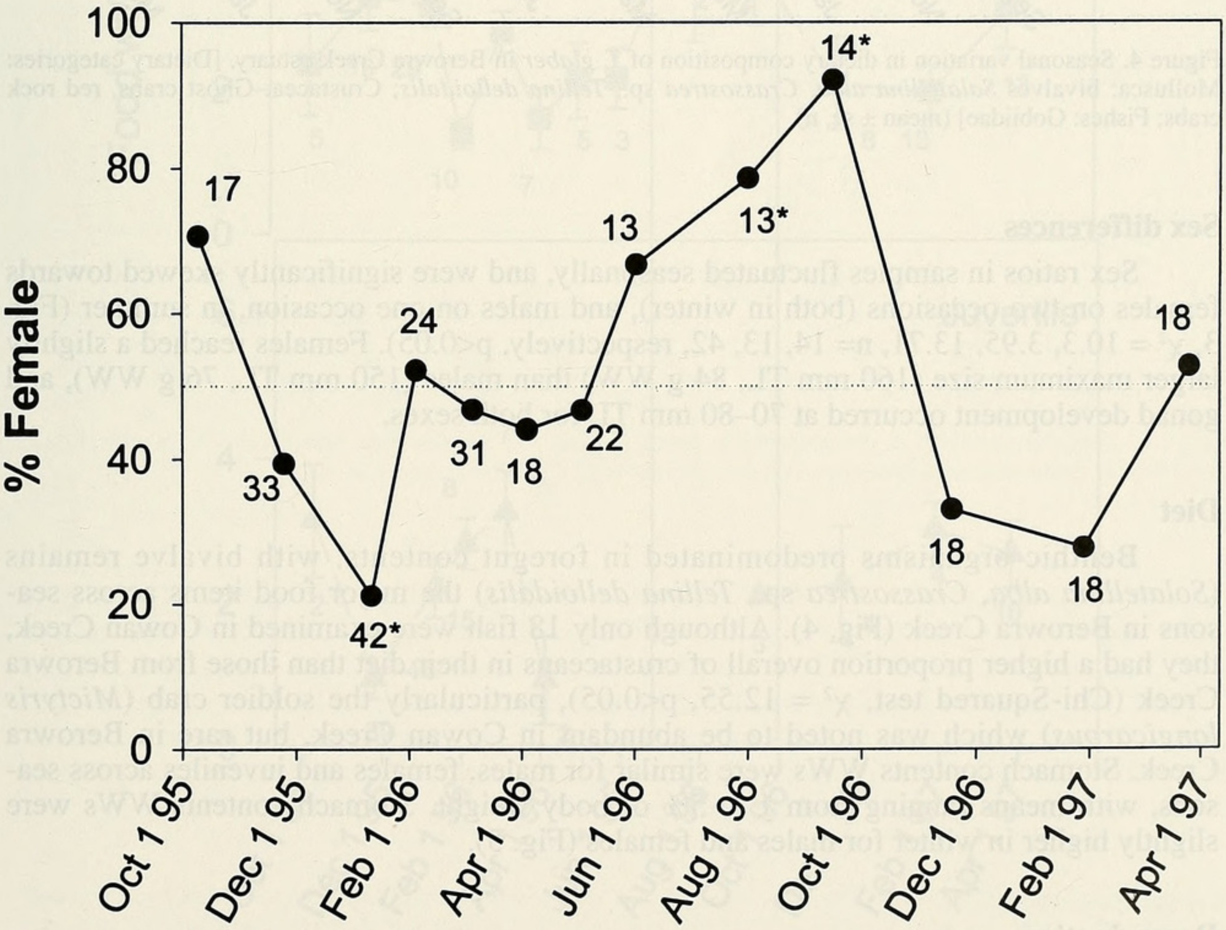

Figure 3. Sex-ratios of *T. glaber* from Berowra Creek estuary between October 1995 and March 1997. Asterisks indicate significant deviations from unity (Chi-squared tests, p<.05, 1:1 male:female, shown by solid horizontal line). Sample sizes indicated.

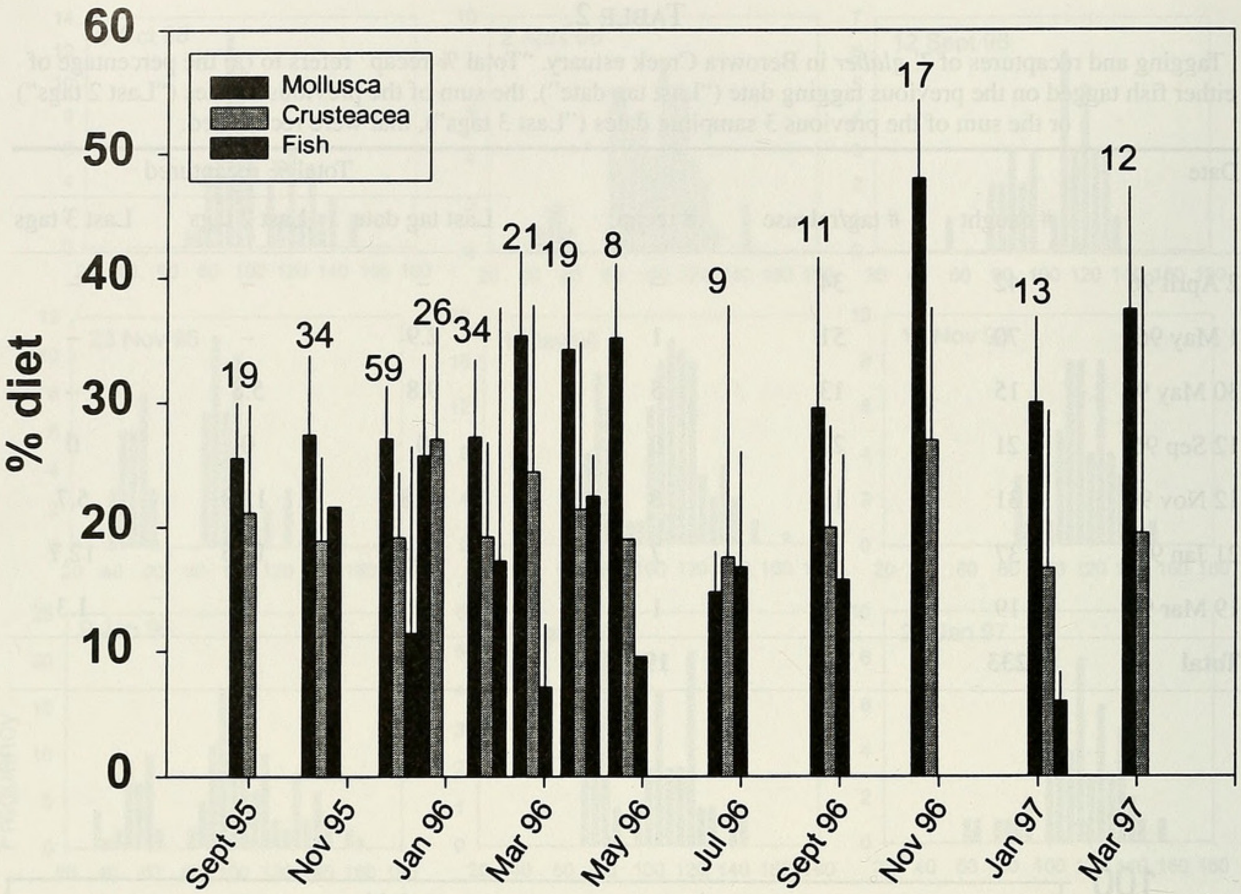

Figure 4. Seasonal variation in dietary composition of *T. glaber* in Berowra Creek estuary. [Dietary categories: Mollusca: bivalves *Salatellina alba, Crassostrea* sp., *Tellina delloidalis*; Crustacea: Ghost crabs, red rock crabs; Fishes: Gobiidae] (mean ± se, n).

Sex differences

Sex ratios in samples fluctuated seasonally, and were significantly skewed towards females on two occasions (both in winter), and males on one occasion, in summer (Fig. 3, $\chi^2 = 10.3, 3.95, 13.71$, n= 14, 13, 42, respectively, $p<0.05$). Females reached a slightly larger maximum size (160 mm TL, 84 g WW) than males (150 mm TL, 76 g WW), and gonad development occurred at 70–80 mm TL for both sexes.

Diet

Benthic organisms predominated in foregut contents, with bivalve remains (*Solatellina alba*, *Crassostrea* sp., *Tellina delloidalis*) the major food items across seasons in Berowra Creek (Fig. 4). Although only 18 fish were examined in Cowan Creek, they had a higher proportion overall of crustaceans in their diet than those from Berowra Creek (Chi-Squared test, $\chi^2 = 12.55$, $p<0.05$), particularly the soldier crab (*Mictyris longicarpus*) which was noted to be abundant in Cowan Creek, but rare in Berowra Creek. Stomach contents WWs were similar for males, females and juveniles across seasons, with means ranging from 1 to 5% of body weight. Stomach contents WWs were slightly higher in winter for males and females (Fig. 5).

Reproduction

For both males and females, gonad weight was related to DW (Males: $r^2 = 0.13$, $p<0.001$, n = 155, Females: $r^2 = 0.45$, $p<.001$, n = 153), and both regressions passed

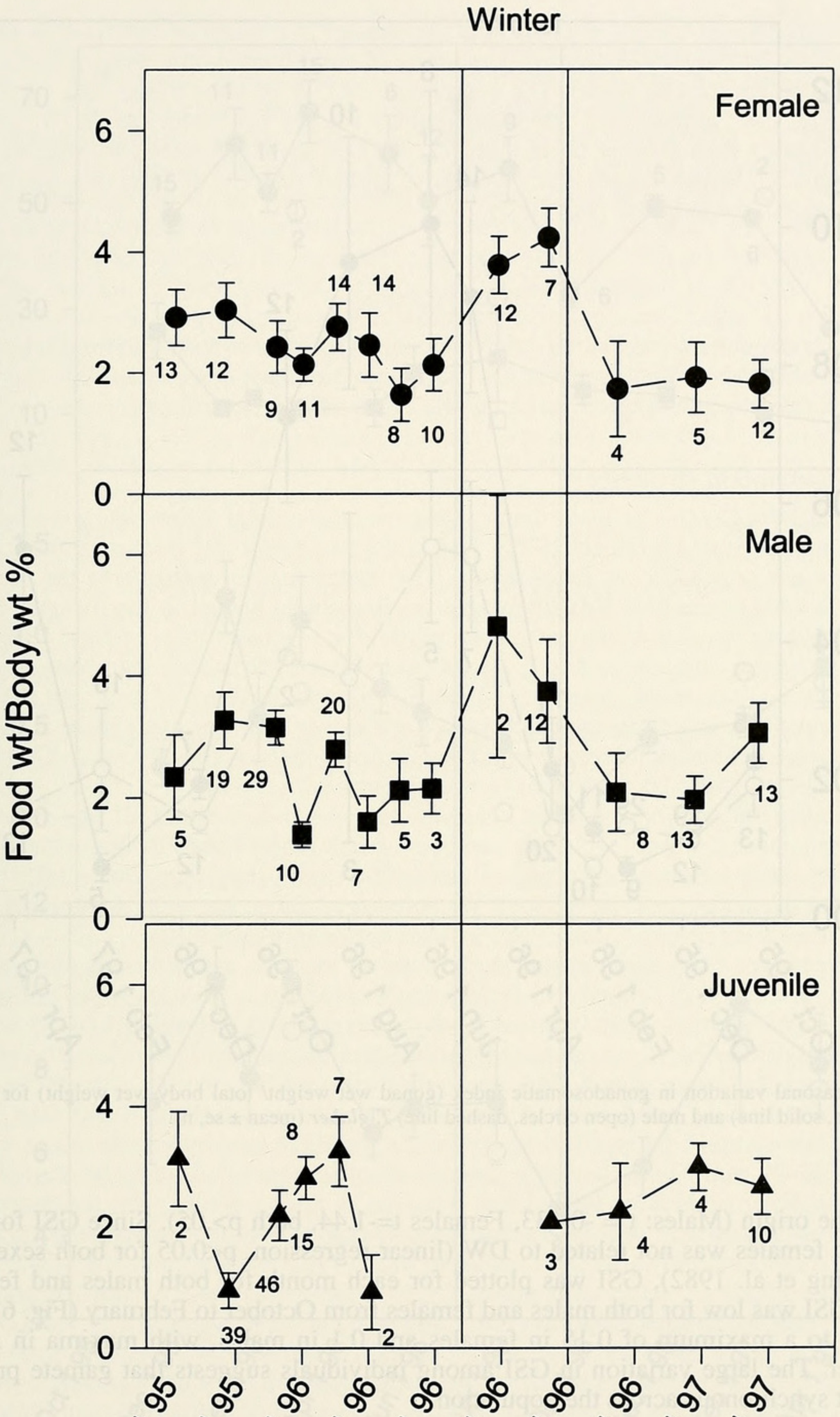

Figure 5. Seasonal variation in foregut contents weight (as a % of body weight) for female, male and immature *T. glaber* in Berowra Creek (mean ± se, n). No juveniles were caught in July. Vertical solid lines delineate winter period.

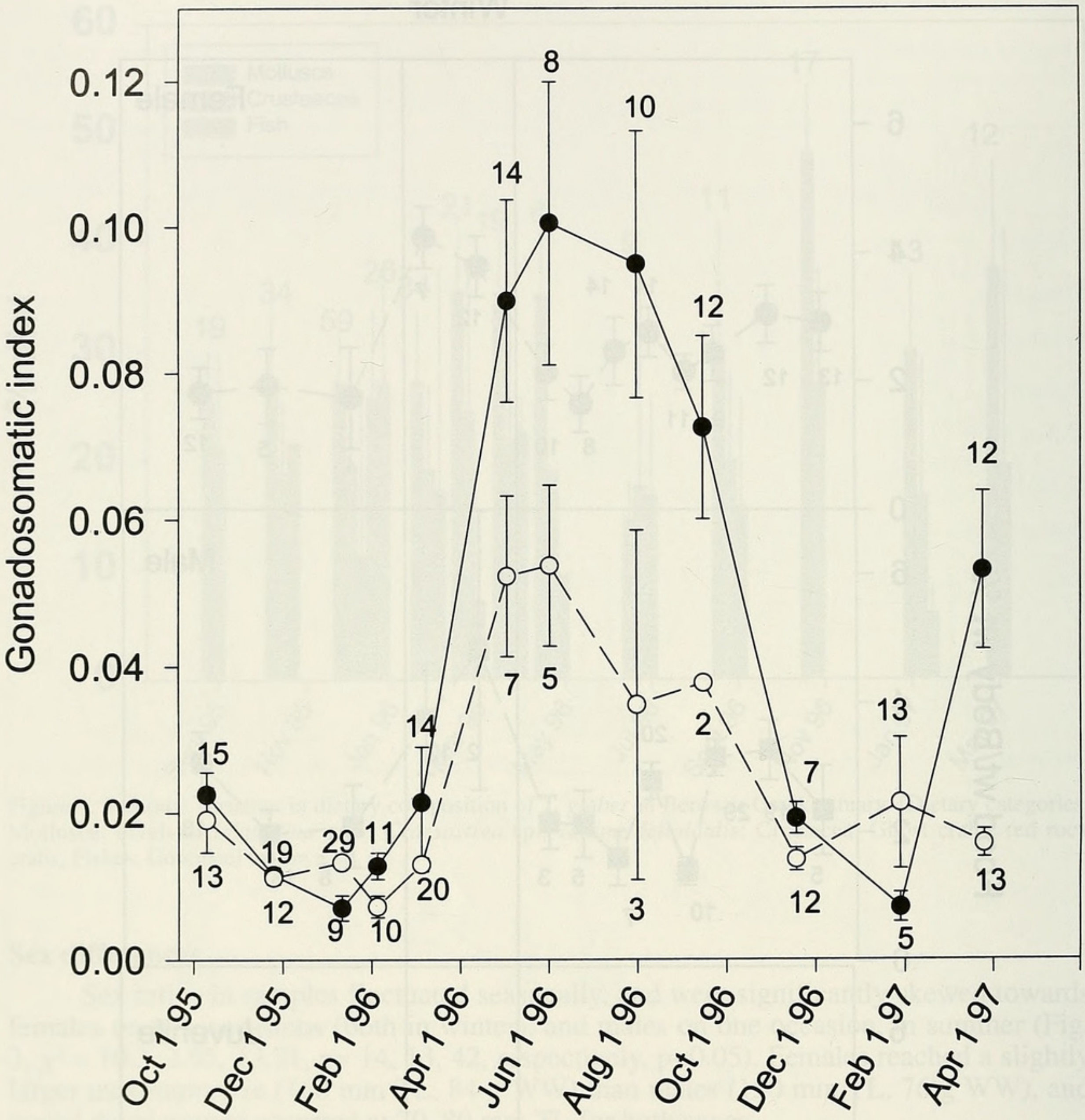

Figure 6. Seasonal variation in gonadosomatic index (gonad wet weight/ total body wet weight) for female (solid circles, solid line) and male (open circles, dashed line) *T. glaber* (mean ± se, n).

through the origin (Males: t = -0.433, Females t=-1.44, both p>.05). Since GSI for both males and females was not related to DW (linear regression, $p<0.05$ for both sexes, see De Vlaming et al. 1982), GSI was plotted for each month for both males and females (Fig. 6). GSI was low for both males and females from October to February (Fig. 6). GSI increased to a maximum of 0.15 in females and 0.1 in males, with maxima in April-September. The large variation in GSI among individuals suggests that gamete production is not synchronous across the population.

Somatic condition

Lipid concentration of the liver of fish in Berowra Creek was higher than that of the muscle tissue at all times at both locations, peaking at a mean of 70% DW in February 1996 (Fig. 7a). This is the time that gonads increase in size (Fig. 6). Muscle tis-

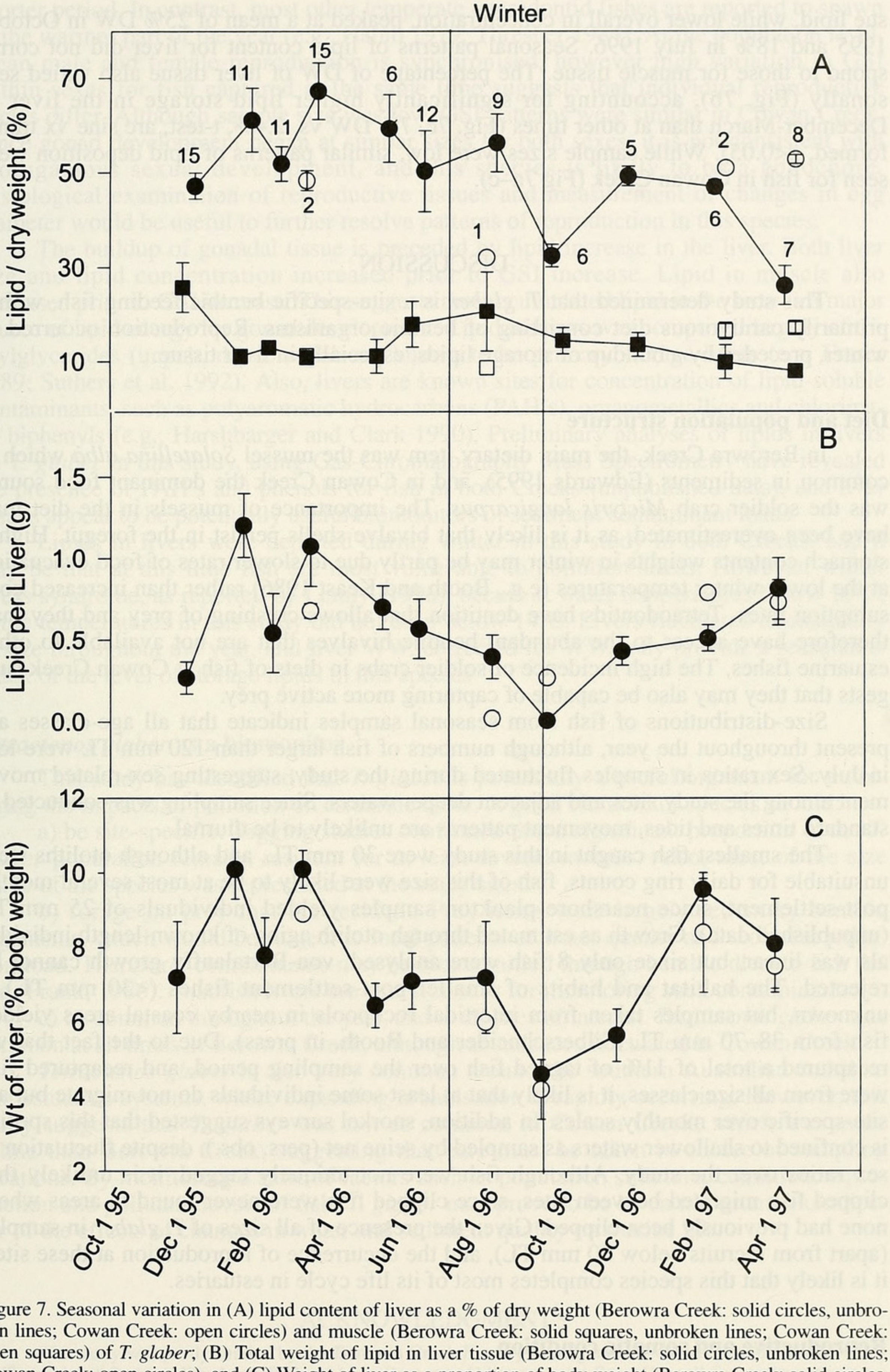

Figure 7. Seasonal variation in (A) lipid content of liver as a % of dry weight (Berowra Creek: solid circles, unbroken lines; Cowan Creek: open circles) and muscle (Berowra Creek: solid squares, unbroken lines; Cowan Creek: open squares) of *T. glaber*; (B) Total weight of lipid in liver tissue (Berowra Creek: solid circles, unbroken lines; Cowan Creek: open circles), and (C) Weight of liver as a proportion of body weight (Berowra Creek: solid circles, unbroken lines; Cowan Creek: open circles). (mean ± se, n indicated, n for both sites indicated in top panel).

sue lipid, while lower overall in concentration, peaked at a mean of 25% DW in October 1995 and 18% in July 1996. Seasonal patterns of lipid content for liver did not correspond to those for muscle tissue. The percentage of DW of liver tissue also varied seasonally (Fig. 7b), accounting for significantly higher lipid storage in the liver in December-March than at other times (Fig. 7c, 7% DW vs. 2.4%, t-test, arc sine $\sqrt{x}$ transformed, $p<0.05$). While sample sizes were low, similar patterns of lipid deposition were seen for fish in Cowan Creek (Fig 7a–c).

DISCUSSION

This study determined that *T. glaber* is a site-specific benthic-feeding fish, with a primarily carnivorous diet consisting of benthic organisms. Reproduction occurred in winter, preceded by a buildup of storage lipids, especially in liver tissue.

Diet and population structure

In Berowra Creek, the main dietary item was the mussel *Solatellina alba* which is common in sediments (Edwards 1995), and in Cowan Creek the dominant food source was the soldier crab *Mictyris longicarpus*. The importance of mussels in the diet may have been overestimated, as it is likely that bivalve shells persist in the foregut. Higher stomach contents weights in winter may be partly due to slower rates of food evacuation at the lower winter temperatures (e.g., Booth and Keast 1986) rather than increased consumption rates. Tetraodontids have dentition that allows crushing of prey and they may therefore have access to the abundant benthic bivalves that are not available to other estuarine fishes. The high incidence of soldier crabs in diets of fish at Cowan Creek suggests that they may also be capable of capturing more active prey.

Size-distributions of fish from seasonal samples indicate that all age-classes are present throughout the year, although numbers of fish larger than 120 mm TL were low in July. Sex ratios in samples fluctuated during the study, suggesting sex-related movement among the study sites and adjacent deeper waters. Since sampling was conducted at standard times and tides, movement patterns are unlikely to be diurnal.

The smallest fish caught in this study were 30 mm TL, and although otoliths were unsuitable for daily ring counts, fish of this size were likely to be at most several months post-settlement, since nearshore plankton samples yielded individuals of 25 mm TL (unpublished data). Growth as estimated through otolith aging of known-length individuals was linear, but since only 8 fish were analysed, von-Bertalanffy growth cannot be rejected. The habitat and habits of smaller post-settlement fishes (<30 mm TL) is unknown, but samples taken from intertidal rockpools in nearby coastal areas yielded fish from 38–70 mm TL (Silberschneider and Booth, in press). Due to the fact that we recaptured a total of 11% of tagged fish over the sampling period, and recaptured fish were from all size classes, it is likely that at least some individuals do not migrate but are site-specific over monthly scales. In addition, snorkel surveys suggested that this species is confined to shallower waters as sampled by seine net (pers. obs.), despite fluctuation in sex ratios over the study. Although fish were not uniquely tagged, it is unlikely that clipped fish migrated between sites, since clipped fish were never found in areas where none had previously been clipped. Given the presence of all sizes of *T. glaber* in samples (apart from recruits below 30 mm TL), and the occurrence of reproduction at these sites, it is likely that this species completes most of its life cycle in estuaries.

Reproductive and somatic condition

The reproductive season appears to be protracted, with high mean GSI from April to September (winter), however individuals may be reproductively-active for a much shorter period. In contrast, most other temperate teteradontid fishes are reported to spawn in the warmer part of the year (e.g., Habib 1979; Thresher 1984). At the population level, mean male and female reproduction is synchronised, however high variation in GSI within sexes for fish captured at the same time suggests that individual reproductive cycles differ. Although sample size was low, GSI patterns were similar in Cowan Creek. Since gonad development began at similar sizes for both sexes, it is not consistent with protogamous sexual development, and this species is likely to be a gonochore. Histological examination of reproductive tissues and measurement of changes in egg diameter would be useful to further resolve patterns of reproduction in this species.

The buildup of gonadal tissue is preceded by lipid increase in the liver. Both liver size and lipid concentration increased prior to GSI increase. Lipid in muscle also increased prior to GSI increase. This suggests that accumulated lipid in the liver is a major source of lipid energy for gonad development. Lipid in livers was comprised largely of tri-acylglycerides (unpub. data), which are known to be primary storage lipids (e.g. Fraser 1989; Suthers et al. 1992). Also, livers are known sites for concentration of lipid-soluble contaminants, such as polyaromatic hydrocarbons (PAH's), organometallics and chlorinated biphenyls (e.g., Harshbarger and Clark 1990). Preliminary analyses of lipids in livers on *T. glaber* in this study, using Gas-Chromatography Mass Spectrometry have revealed the presence of PAH's and phenols for fish in both Creeks (unpublished data), and liver assays appear to be potentially useful biomonitors of sediment contaminant loads.

Lipids in livers were depleted during winter in this study in both Creeks, and it may be that at this time fish are most vulnerable to pollution stress. However, winter stress syndrome, as reported by Lemly (1996), occurs at water temperatures below 10°C, while temperatures in this study did not drop below 14°C. Livers fluctuated dramatically in size throughout the year, and liver WW, corrected for WW, may provide a reasonable index of the level of storage lipids in this species

Tetractenos glaber as a biomonitor

This study has identified that *T. glaber* is potentially a useful organism for monitoring the impacts of pollution on estuarine biota. A good biomonitor species should:

a) be site-specific. Tagging studies confirmed that a significant proportion of the *T. glaber* population remains resident for at least several months. Also, most of the size range of the species was represented at the study sites.

b) forage on prey that are resident. Prey items were largely sedentary benthic organisms, which would be subject to local patterns of water quality and sediment pollution load. Therefore, contaminants may be biomagnified through benthic diets to fish tissue (Frank, 1986). Toadfish livers are useful tissues for monitoring such contaminants.

c) be common throughout the year, and at all sites of interest. *Tetractenos glaber* was common at all times in Berowra Creek, although abundances fluctuated in Cowan Creek.

Tetractenos glaber is also very common around the southern Australian coastline and estuarine systems, where expanding urbanisation is rapidly occurring. However, this study suggests that *T. glaber* was not as common in Cowan Creek, which was more saline than Berowra Creek, suggesting that care must be taken in choice of sampling locations. By monitoring a suite of factors such as population structure, condition, reproduction and pollutant levels of fish, *T. glaber* may prove to be be an important bioindicator of the effects of changes in water and sediment quality on marine life.

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