

Reproductive Biology of Estuarine Pufferfish, *Marilyna pleurosticta* and *Tetractenos hamiltoni* (Teleostei: Tetraodontidae) in Northern New South Wales: Implications for Biomonitoring

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Because of their broad distribution, site fidelity and long lifespan pufferfish (family Tetraodontidae) show potential as biomonitors of estuarine water quality, and as understanding the reproductive cycle is crucial to interpreting variations in contaminant loads in the tissues of biomonitors, we investigated the spawning season, length at maturity and body condition of two small sympatric pufferfish species (*Marilyna pleurosticta* and *Tetractenos hamiltoni*), in the Richmond Estuary, NSW. *M. pleurosticta* spawned in spring while *T. hamiltoni* spawned in winter. Female and male *M. pleurosticta* matured at a similar size (50% mature at 90 mm total length). In *T. hamiltoni* there was a more marked difference in size at 50% maturity, with males maturing at 80 mm and females at 110 mm TL. From the high values for hepatosomatic index (HSI) and its strong inverse relationship with gonadosomatic index (GSI) we inferred that lipid reserves in the liver play an important role in gonad maturation and spawning. Somatic condition factor (K_s) also varied, albeit less so, throughout the year, suggesting that body fat and muscle play lesser roles in providing energy for reproduction. Seasonality of liver lipid content and different spawning seasons have important implications for designing sampling strategies using these fish, especially when monitoring lipophilic contaminants.

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INTRODUCTION

Pufferfish (Family Tetraodontidae) are common in tropical, subtropical and temperate estuaries (Bell et al. 1984; Hindell and Jenkins 2004), occurring around the Australian coast and throughout the full range of estuarine salinities from freshwater to marine conditions (Gomon et al. 2008; Allen 2009). Whilst they are potentially susceptible to being caught in unsustainable numbers as trawl by-catch (Stobutzki et al. 2001), few are of conservation concern in Australia. Tetraodontids show potential to be used as biomonitors of estuarine contamination because they are widely abundant, long-lived, appear to remain within a small home range and are carnivorous

(Booth and Schultz 1999; Alquezar et al. 2006; Mat Piah 2011). Despite this, there are very few studies of the biology of estuarine pufferfish (NSW Department of Primary Industries 2006).

Reproductive processes may influence the storage, mobilisation and transfer of lipids (Merayo 1996; Alonso-Fernandez and Saborido-Rey 2012) and hence the partitioning of lipophilic contaminants among different tissues (Fletcher and King 1978), so the reproductive cycles and changes in condition of any species to be used as a biomonitor should therefore be well understood. A species on the west coast of Australia, *Torquigener pleurogramma*, is a broadcast spawner during summer and sexual maturity is reached at two years of age (Potter et al.

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1988). A similar species in New Zealand *Contusus richiei* also matures at two years old and spawns in summer (Habib 1979) whereas the smooth puffer *Tetractenos glaber* in the Sydney region spawns in winter (Booth and Schultz, 1999). The reproductive biology of other tetraodontids, other than the few large pelagic species used commercially for human consumption (e.g. Sabrah et al. 2006) has not been documented.

The balance between the metabolic demands for energy and the quantity and quality of food intake determines the amount of energy that can be stored as lipids to fuel growth and reproduction and can be indicated by several indices. Some commonly used indirect measures of a fish's energy status are Fulton's condition factor K , which is an index of the body weight of a fish relative to its length and the hepatosomatic index (HSI), which is a measure of the relative weight of the liver, a major energy store especially in non-fatty fish (McPherson et al. 2011). In pufferfish, the liver is large and easily dissected from other organs. Condition factor indices that use gutted (somatic) weight (K_r) (e.g. Encina and Granado-Lorencio 1997) can provide a better indicator of changes in the food reserves stored in muscle and body fat than indices using total weight, especially in light-framed fishes such as puffers where variations in large organs such as gonad and liver and gut fullness can mask changes in body fat and muscle (McPherson et al. 2011). At high K_r values, excess energy results in accumulation of fat and oil reserves and little demand on protein for energy production, resulting in greater muscle development. At low K_r values, the metabolic energy demands have depleted lipid reserves and are supplemented by catabolism of proteins, resulting in reduced muscle development.

In preparation for spawning HSI may decline during gamete development as lipid reserves are reduced (Htun-han 1978). Somatic condition may also reduce in this time if somatic fat deposits or protein are catabolised during gametogenesis or for spawning migrations (Htun-han 1978). After spawning HSI gradually increases as energy reserves are restored prior to commencement of the next gametogenic cycle (Htun-han 1978). This cycle is dependent on food intake being sufficient to allow reserves to increase between spawning events. If not, fecundity, egg size or yolk content will be reduced in the subsequent spawning (e.g. Burton 1994). The immediate post-spawning period is also generally the time of most rapid somatic growth (Chellapa et al. 1995).

Our study examined the reproductive cycles and condition indices of two pufferfish species commonly found in tropical and sub-tropical Australian estuaries,

Marilyna pleurosticta and *Tetractenos hamiltoni*. These two species commonly occur from northern New South Wales and Queensland (Grant 1987; Edgar 2000), north to Papua New Guinea (Coates 1993), where they inhabit a broad range of habitats from near the mouth of the estuary to its upper low-salinity reaches. If these two widespread species were to be used as biomonitors of estuarine water quality then any differences between the reproductive cycles of the species would need to be considered when choosing the species and time of year to sample. The specific objectives of this study were therefore to quantify the spawning season, and length at 50% maturity of *M. pleurosticta* and *T. hamiltoni*, to determine if seasonal variations of body condition indices occur and differ in *M. pleurosticta* and *T. hamiltoni* and to quantify the relationship between body condition indices and spawning activity of the fish.

MATERIALS AND METHODS

Study area and sampling procedure

Fish were captured each month from March 2008 to March 2010 in a tributary of the Richmond River Estuary, New South Wales, Australia (Fig. 1). Fishes were collected as they returned from foraging over intertidal mangrove forests by setting 12 fyke nets with a mesh size of 12 mm, entrance radius 30 to 45 cm and wing length up to 5m in tidal channels on the ebbing tide. Nets were set on daylight spring high tides and retrieved as they were exposed by the falling tide. After capture, the fish were euthanised using 1 ppt solution of Benzocaine (ethyl-p-aminobenzoate) in water from the capture site, transported on ice packs and frozen within 2 hours of capture.

In the laboratory, thawed fish were measured from snout to distal edge of the caudal fin (total length, TL to the nearest 1 mm). Total weight (TW to the nearest 0.1g) was measured using a pan balance (Mettler Toledo PL3002). Gutted carcasses were weighed on the same balance to provide the somatic weight (SW). Liver and gonad tissues were removed and weighed (LW and GW to the nearest 0.001g) using an analytical balance (Mettler Toledo AL204)..

Reproductive biology analysis

Fish gender was determined using a gonad visual census, in which testes appeared as smooth-textured and ivory-white in colour and ovaries pink to orange in colour with a granular texture of developing oocytes within. Fish in which the gonads were small, thin and transparent and unable to be confidently

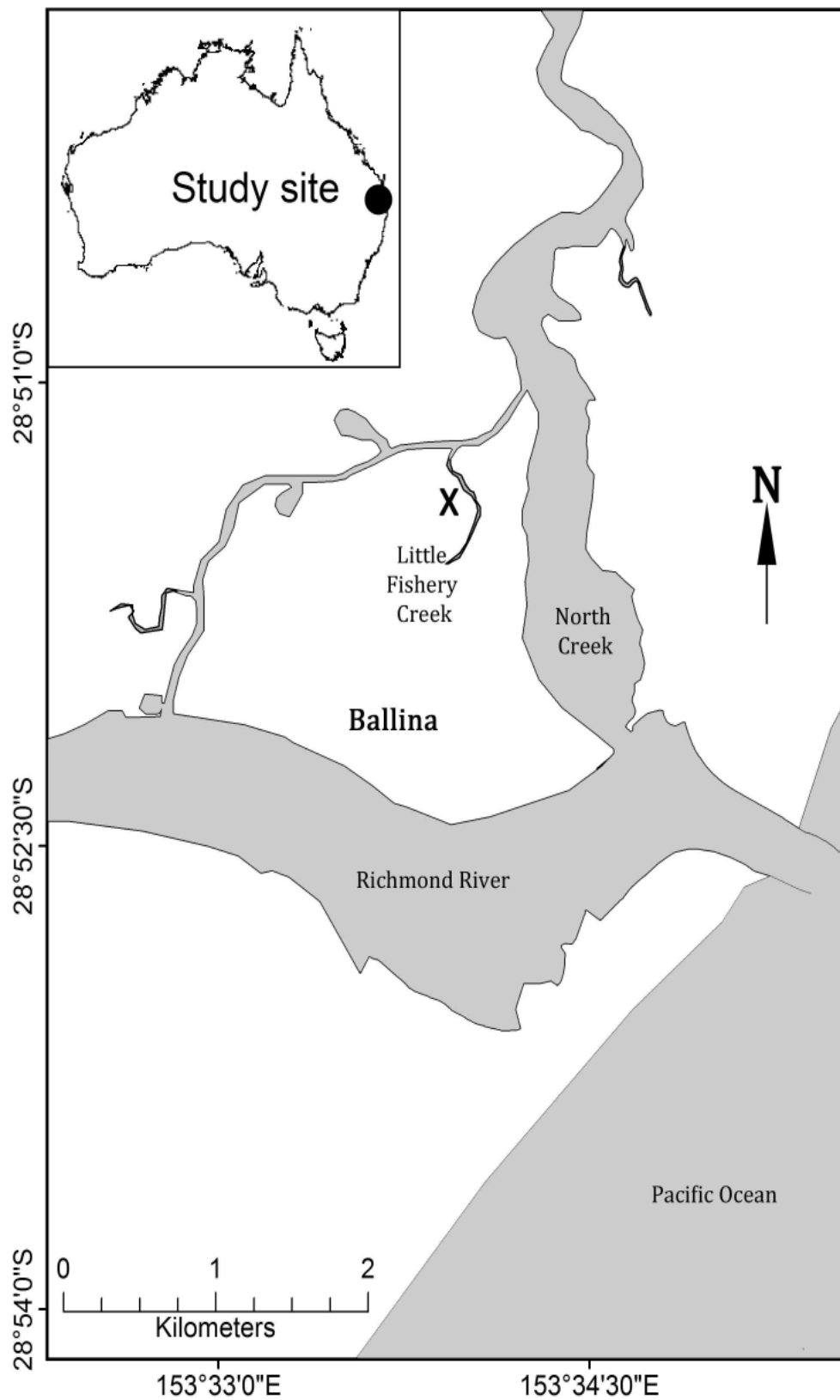


Fig. 1. Location of study site (X) in Little Fishery Creek, a tributary of the Richmond River estuary at Ballina, NSW (base map courtesy of D. Maher, Center for Coastal Biogeochemistry, Southern Cross University).

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assigned to male or female were categorised as immature.

A total of 358 gonads from *M. pleurosticta* and 89 gonads from *T. hamiltoni* were examined. Maturity stages were determined macroscopically and were classified as either I, immature; II, developing; III, spawning-capable; IV, spent/resting (= regressing/regenerating) (modified from Brown-Peterson et al., 2011). The spawning season was determined by following the changes in proportions of the three non-immature stages on a seasonal basis and by following monthly changes in mean gonadosomatic index (GSI), where $GSI = 100 * GW/TW$. Elevated GSI in mature fish indicates that they are approaching spawning season and a rapid reduction in GSI indicates that spawning has recently occurred.

Length at maturity was determined as the length at which 50% of individuals were at maturity stages III or IV, during the spawning season. The length at 50% sexual maturity (L_{50}) was estimated by fitting a logistic model to the combined percentage of fish with maturity stages III and IV in each 1 cm size class (Rogers et al., 2009). The logistic curve was fitted by minimising the sum of squares using the Solver 'add-in' function in Excel (Microsoft Corporation, 2007).

Body condition and HSI analysis

Condition factor index, K_r and hepatosomatic index (HSI) were calculated as indirect indices of energy status. The two parameters were estimated as follows (McPherson et al. 2011):

- a) $HSI = 100 \times LW (g) / SW (g)$
- b) $K_r = 100 \times SW (g) / FL^3 (cm)$

RESULTS

Maturity stages

Catch rates for *T. hamiltoni* were much lower than for *M. pleurosticta*, especially for males, so maturity stages were pooled for each season (three-month intervals). Both testes and ovaries of *M. pleurosticta* showed a seasonal progression of developmental stages, culminating in a switch from a majority of fully mature fish in spring to a majority of spent and resting individuals in summer (Fig. 2). For *T. hamiltoni* the pattern is less clear but most spent or resting individuals of both sexes were caught in spring and summer whereas spawning-capable fish were more common in autumn and winter. Fish commencing a new gametogenic cycle were most common in summer (Fig. 2).

Gonadosomatic Index

Male and female *M. pleurosticta* both followed similar patterns of monthly mean GSI (Fig. 3a). The pattern was consistent for both years, elevated from September and decreased in December. It is assumed that the GSI value probably peaked in November but no fish were captured in this month in either 2008 or 2009 presumably having left the mangrove habitat to spawn elsewhere in the estuary or at sea. For *T. hamiltoni*, GSI of females in both 2008 and 2009 showed a double peak with a maximum in April, declining in May and increasing to a second larger maximum in June or July (Fig. 3b). Catch rates for males were too low to display meaningful patterns, although they too peaked in April of both years.

Length at maturity

Fifty percent of female *M. pleurosticta* reached sexual maturity at 89 mm and for males 50% reached sexual maturity at 92 mm (Fig. 4a, b). At the total length of 120 mm, all females were sexually mature while all males above 130 mm were mature. In contrast, male *T. hamiltoni* reached sexual maturity at smaller size than females. Fifty percent of males were sexually mature at 70 mm and by the total length at 80 mm all males were sexually mature whereas 50 percent of females attained sexual maturity at 83 mm and all females were sexually mature at 110 mm (Fig. 4c, d).

Seasonal changes in GSI, K_r and HSI

The ranges of seasonal mean condition factor indices K_r were very similar for both sexes of both species. For each species the seasonal patterns of average HSI were similar for the two sexes and have been pooled for analyses, but the patterns for the two species are very different to each other (Fig. 5). The seasonal mean hepatosomatic and gonadosomatic indices have an inverse relationship to each other in both species. In *M. pleurosticta*, there is little seasonal change in K_r . However, both K_r and GSI values were highest when HSI was decreasing. In *T. hamiltoni*, the pattern of seasonal mean HSI is similar to K_r while the GSI was peaking in winter when the HSI was at its lowest.

DISCUSSION

Spawning season

Despite the superficial similarity of these two species and their similar habitats, they display quite different reproductive cycles. While *M. pleurosticta* spawns in late spring, *T. hamiltoni* spawns in winter, possibly with a split spawning in early and late

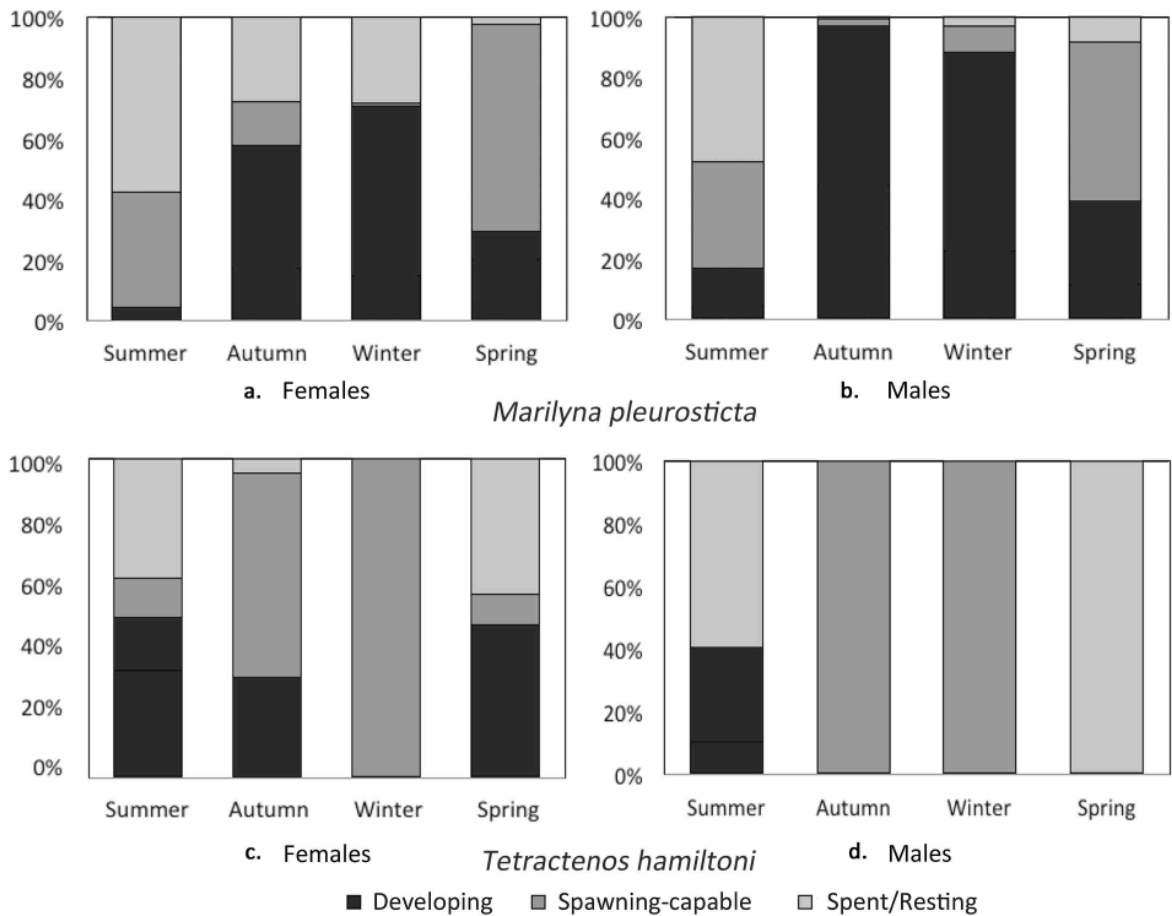


Fig. 2. Seasonal proportions of the three mature categories of gonad development for females (left) and males (right) of *M. pleurosticta* (top) and *T. hamiltoni* (bottom).

winter. The reproductive cycle of *M. pleurosticta* in this study was different to other tetraodontid species reported, most of which spawn in summer (Habib 1979; Potter et al. 1988; Sabrah et al. 2006). However, the spawning season for *T. hamiltoni* is very similar to the closely related *T. glaber* in the Hawkesbury estuarine system, which also spawns in winter (Booth and Schultz 1999).

Spawning location

The consistent lack of captures of both species in November and December of both years followed by an increase in capture rates in subsequent months suggests that both species probably leave the mangrove systems at this time. Tag returns (Mat Piah 2011) suggest that at least some adults subsequently return to the same channel system. Gonadosomatic indices indicated that the majority of *M. pleurosticta* caught prior to November were mature and the largest proportion of stage IV (spent/resting) individuals

were captured in the months shortly after November, indicating that the absence of that species coincided with spawning. This finding is similar to the studies in Swan River estuary (Potter *et al.* 1988), where mature *T. pleurogramma* migrate out of the estuary to spawn in shallow coastal waters between October and January. At these times, large schools of this species have often been observed passing out to sea by fishers (Potter et al. 1988). *T. hamiltoni* were also not caught in November and December of both years. However, at this time their declining GSI suggests that, while this species also leaves the mangroves in early summer it is for reasons other than a spawning migration.

Length at maturity

Although gonad development and subsequent spawning may depend on various environmental stimuli, individuals must reach a certain age or size before they are capable of spawning (King 2001),

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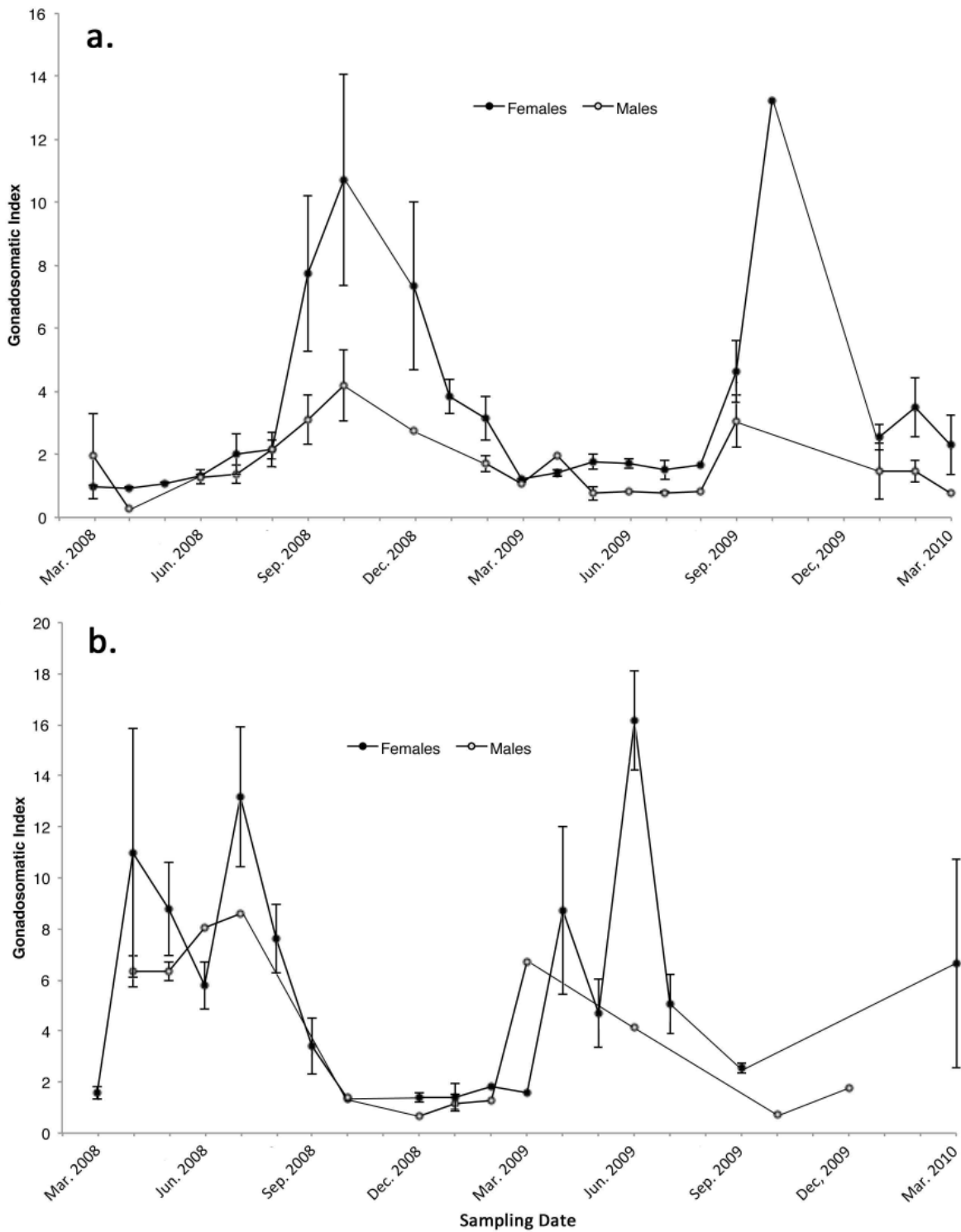


Fig. 3. Monthly mean (\pm S.E.) of Gonadosomatic Index (GSI) of females and males of (a) *M. pleuroticta* and (b) *T. hamiltoni*.

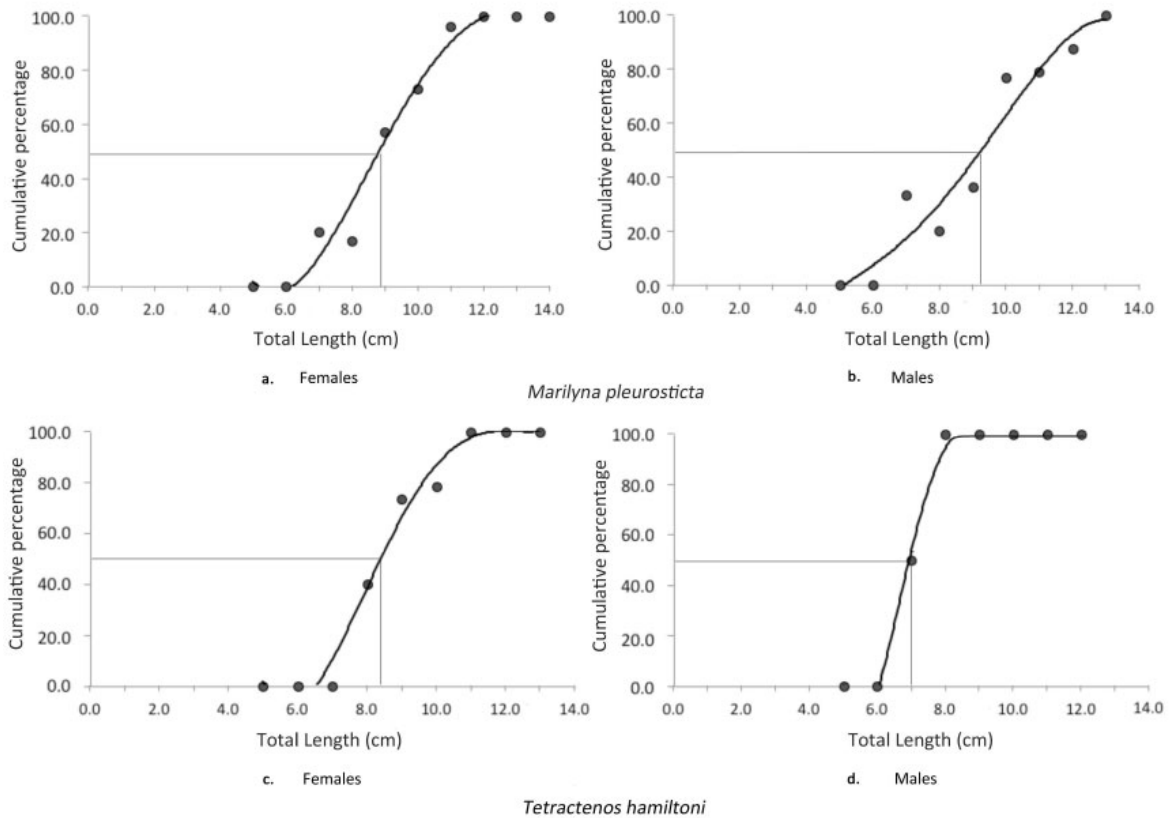


Fig. 4. Length at maturity for *M. pleurosticta* (a and b) and *T. hamiltoni* (c and d). Lengths at which 50% of individuals are mature are indicated.

and dependency of maturation to the age or length is strongly linked to growth and is also regulated by the water temperature and feeding success (Yoneda et al. 2001; Takemura et al. 2004).

In this study, female *M. pleurosticta* reached sexual maturity at a similar size to males, whereas, male *T. hamiltoni* start to mature at a much smaller size than females. It is not known if these size differences are due to different growth rates or different ages at maturity. Preliminary unvalidated age estimates from otolith growth checks suggest that age at maturity for both species may be as high as 10 years (Mat Piah 2011). The length at maturity in pufferfish in this study was similar to that of *U. richiei* that matures at 7.5 to 11.6 cm (Habib 1979) while Sabrah et al. (2006) determined that the large oceanic species *Lagocephalus sceleratus* in the Gulf of Suez reached maturity at a length of 42.1 cm for males and 43.3 cm for females.

Relationship between energy storage and reproductive activity

Many studies calculate K using total weight including the gonads. In the case of puffers

that have relatively light bodies with reduced skeletal components and large gonads and liver, such formulae would have been strongly influenced by individual organ development and variable gut fullness, potentially masking changes in muscle and fat body mass. For this reason, K_r was calculated by excluding visceral weight from the numerator. The cyclical variation in hepatosomatic index while condition changed much less suggests a central role for the liver as a source of lipid and metabolic energy fuelling gamete production.

There is usually a direct correlation between hepatosomatic index and body condition index, and an inverse correlation of these factors to gonadosomatic index (Htun-han 1978). In this study HSI displayed a strong inverse correlation with GSI in both species, but K_r in *M. pleurosticta* showed no correlation with either of the other two indices. However, in *T. hamiltoni* K_r showed a positive relationship with HSI. The increase in GSI during the period of gonad maturation is mainly due to the deposition of large amounts of proteins and lipids in the developing eggs and spermatozoa (Htun-han 1978). Part of this material comes directly from ingested food

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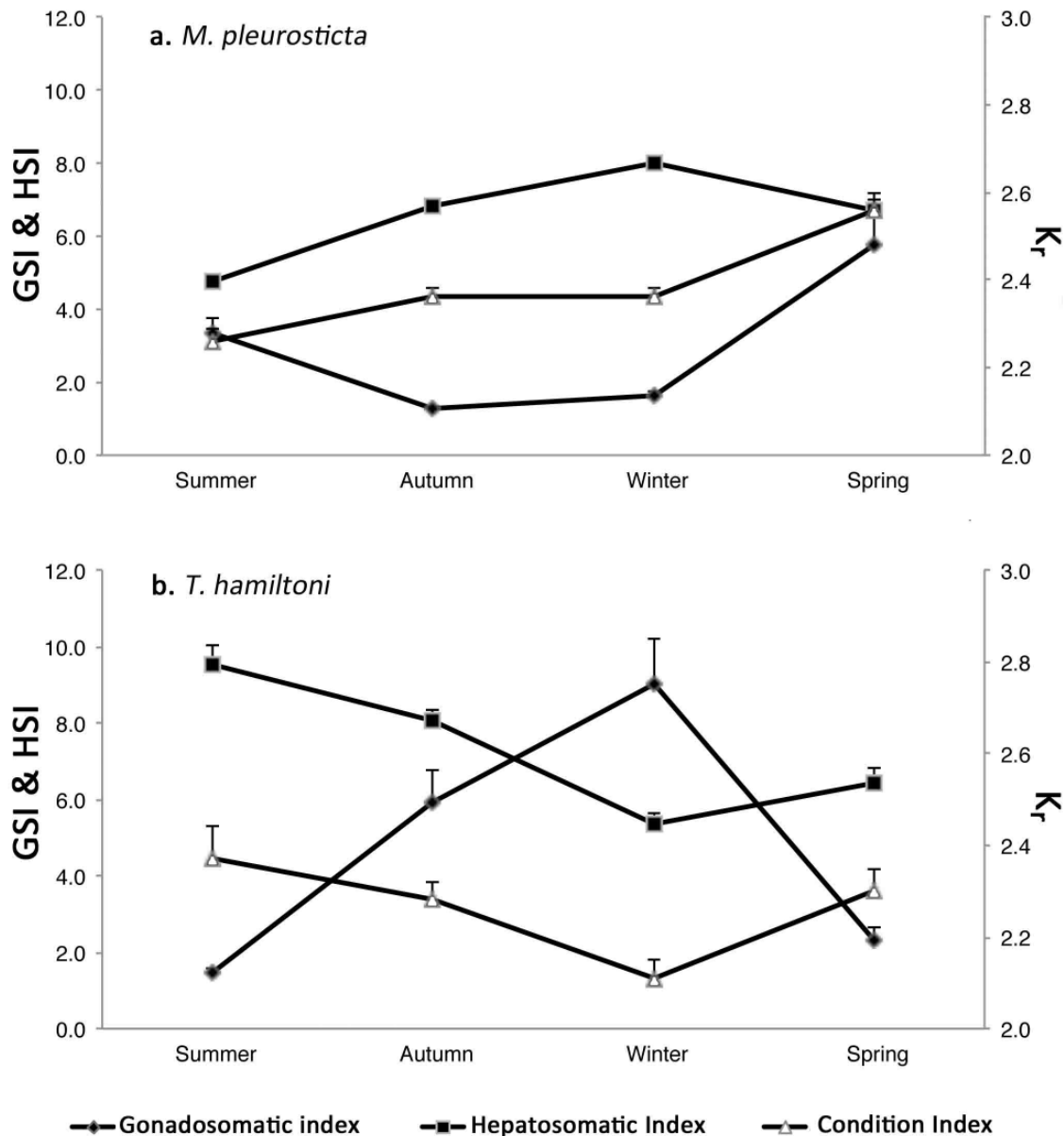


Fig. 5. Seasonal relationships between mean hepatosomatic Index (HSI), condition factor (K_r) and gonadosomatic index (GSI) of (a) *M. pleurosticta* and (b) *T. hamiltoni* (sexes combined).

but a major proportion comes from reserves of food deposited during the active feeding season in organs such as liver and muscles (Larson 1974). It is therefore reasonable to expect that the weight of liver and muscle would reflect the cycle of accumulation and utilization of these energy reserves.

Summer is important for both species because it is the time of greatest growth after spawning as energy is going into somatic tissue rather than gonads. The increase in HSI also indicates an excess of energy intake over immediate needs in this season. This would

be a critical time for the species and feeding success at this time could affect fecundity in the next spawning. A flood in January 2008 resulting in low dissolved oxygen and low pH for several months afterwards may have substantially affected these, and potentially other species during the first year of this study by reducing feeding opportunities during this critical period and may explain the interannual differences in the proportions of mature and spent gonads, which were more common in samples from 2009 than in 2008.

The strong inverse relationship between HSI and GSI suggests that lipid storage in the liver is critical to reproductive success in both species. With mean HSI values of between 6 and 10% of somatic weight, the liver of these small pufferfish is unusually large for the size of the fish. For comparison, other fish species collected from the Richmond River in August 2004 (Bucher, unpublished data) produced mean HSI values for mature bream *Acanthopagrus australis* of 0.7 percent, luderick *Girella tricuspidata* of 1.0 percent and sand whiting *Sillago ciliata* of 1.8 percent. Mean GSI values of pufferfish at full maturity (8-9) are also large compared to mature bream *Acanthopagrus australis* of 4.0, luderick *Girella tricuspidata* of 7.6 and sand whiting *Sillago ciliata* of 2.1 (Bucher, unpublished data).

There was a different pattern in the relative dynamics of HSI, K_r and GSI between these two species. *Marilyna pleurosticta* starts reserving lipids during the pre-spawning period, for use during spawning. The same pattern was found in Irish Sea plaice, *Pleuronectes platessa* (Wingfield and Grimm 1977), where HSI was also highest in the pre-spawning period and lowest in the post-spawning period. The reduction in HSI and K_r over the reproductive season may be explained by mobilization of lipid reserves, and especially vitellogenin (Vg), a lipophosphoprotein yolk-precursor synthesized by the liver (Maldonado-Garcia et al. 2005). Somatic condition mirrored the pattern of HSI in both species, indicating that lipids stored outside the liver are also important in fuelling the reproductive process.

In *T. hamiltoni*, HSI and K_r declined during pre-spawning presumably for gonad maturation. This pattern is similar to that of smooth pufferfish *Tetractenos glaber* in the Sydney region (Booth and Schultz 1999). In a study of liver weights of brook trout Larson (1974), suggested that the decrease in liver weight during pre-spawning season was due to the passage of materials from the liver to the gonads and concluded that weight changes of the liver plays an important role in gonad maturation.

There was not only a change in weight but also a change in colour and texture of the liver with different stages of the gametogenic cycle. The pre-spawning liver of both species was firm and pale while the post-spawning liver was soft, dark and flaccid. This supports the concept that lipids in the liver have been used for the spawning process. Rossouw (1987) reported that the liver colour was in synchrony with the variation in the total liver lipid content in both sexes of sand sharks. He found that the higher liver lipid concentration in the liver, the lighter the livers become in appearance.

High values for hepatosomatic indices and their strong inverse relationship with gonadosomatic indices demonstrate that mobilisation of lipid stores in the large liver is important for fuelling gametogenesis and low feeding success during periods of high river flow at critical times of the year could therefore potentially severely affect spawning success. The large variability in lipid content of the liver has implications for tissue loads of lipophilic pollutants if these ubiquitous, long-lived fishes are to be used as biomonitors of estuarine pollution. The differences in timing of lipid mobilisation for gonad development also mean that the species being used for a biomonitoring program will determine the timing of sampling.

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