

University of Tasmania

Institute of Marine and Antarctic Studies

**Reproduction, Growth and Population
Dynamics of Estuary Perch (*Percalates colonorum*) in
the Arthur River, Tasmania.**

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Declaration of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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Abstract

This project examined stock structure, population size, reproductive biology, growth and the suitability of using scales and anal fin spines as a non-destructive ageing hard part in the last remaining estuary perch (*Perca latipes*) population in Tasmania, within the Arthur River. Reproductive stage and gonadosomatic indices (GSI) indicated that spawning occurred in summer when temperatures were between 15 - 18°C and salinities were 20 – 35ppt. Both ovarian and batch fecundity were size dependent and ranged between 897, 367 – 2, 458, 425 (ovarian fecundity) and 100, 227 – 228, 200 oocytes (batch fecundity). The smallest mature male measured 20 cm FL (4 yrs old) while the smallest mature female measured 29 cm FL (6 yrs old) respectively. Mature (stage 3) females displayed a tri-modal distribution of oocytes indicating a group synchronous, multiple spawning strategy, with the number of potential batches varying between 6 -14 spawning events in a season. Age and readability was compared between sectioned otoliths, fin spines and mounted scales. Age estimates in both scales and fin spines were significantly underestimated and these structures were not suitable to age estuary perch. Estuary perch were long lived with maximum ages of 19 yrs for females and 34 yrs for males (otolith based). Age structures were similar between sex and showed strong, weak and missing cohorts with ages between 12-14 yrs dominating the catch (68%) and a smaller, secondary mode of fish between 4-8 yrs (27%) was also evident. In contrast to age structures, length frequency distributions were markedly different with females ruling the larger size classes between 34 and 45 cm. Growth modelling from raw and back-calculated size-at-age data suggested that growth is relatively slow however, female growth models had larger L_{∞} and K values compared to males. Mark-recapture tagging produced an adult population estimate of 1, 594 fish. Considering these results of slow growth, long lived, episodic recruitment and the historic decline of estuary perch stocks in Tasmania, it is recommended that the current no-take policy should continue.

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List of acronyms and symbols

ANOVA	analysis of variance
IAPE.....	index of average percent error
AoRSS.....	analysis of residual sum of squares
CF.....	correction factor
Df.....	degrees of freedom
DO.....	dissolved oxygen
F.....	F-value
GSI.....	gonadosomatic index
HSD.....	honest significant difference
K	Von Bertalanffy growth coefficient
L_{∞}	Von Bertalanffy theoretical asymptotic length
MIA.....	marginal increment analysis
NSW.....	New South Wales
P.....	P-value
POF.....	post ovulatory follicles
SE.....	standard error
T_0	Von Bertalanffy theoretical age at length equal to zero
VB.....	Von Bertalanffy

1.0 Introduction

1.1 Overview

The Percichthyidae family of the infraclass Teleostei is restricted to temperate basses and perches found in Australia and South America (Ruzzante et al. 2006, Knight et al. 2009, Smith et al. 2011). Traditionally, percichthyids live in freshwater environments although evolutionary changes have resulted in adaptations to marine and estuarine salinities (Trnski et al. 2005, Ruzzante et al. 2006, Walsh et al. 2010). Percichthyids live in sensitive environments and often occupy niches as top order consumers, illustrating their importance in balancing community structure (Walsh et al. 2011). Certain members of the family are important for recreational and commercial fishing and are future aquaculture prospects (Alam and Frankel 2006, Walsh et al. 2010). In Australia, populations of percichthyids have declined significantly with all four species of freshwater cod listed as either endangered or threatened and four of the six pygmy perch species listed as endangered or vulnerable (Lintermans et al. 2005, Knight and Arthington 2008). Factors resulting in the observed declines include anthropogenic activities such as overfishing and reduced spawning opportunities from habitat degradation (Schwartz et al. 2005, Tonkin et al. 2010).

Estuary perch (*Percalates colonorum*; previously *Macquaria colonorum*), together with the closely related Australian bass (*Percalates novemaculeata*) make up the only two catadromous percichthyids in the world (Walsh et al. 2011). Estuary perch is a prized recreational angling species which is popular for its aggressive fight on light tackle and its exceptional table qualities (Walsh et al. 2011). As estuary perch is a catadromous species they aggregate to spawn in lower inter-tidal waters before dispersing and inhabiting either

riverine or upstream freshwater environments post-spawning (McCarragher and McKenzie 1986, McDowall 1996, van der Meulen et al. 2014). The northern distribution of estuary perch covers rivers from central New South Wales (NSW) and extends down to Victoria. Their western range is limited to the Murray River in South Australia while the most southern stocks are found in Tasmania (McCarragher and McKenzie 1986, Walsh et al. 2010).

As Tasmania's largest native freshwater fish, estuary perch have been positively identified and reported from the Ansons and Arthur Rivers. However, anecdotal reports from recreational fishers suggest that around the mid 1950's estuary perch inhabited many other northern Tasmanian estuaries (Personal comment: Michael Haley). Unfortunately there is no evidence that these populations still exist today and the Ansons River population has not been evident since 1980 (McCarragher and McKenzie 1986). As such it appears that, the species has become locally extinct in all but one Tasmanian estuary, with their current distribution being limited to the Arthur River on the West Coast (DPIPWE, 2014). It is currently unknown whether the Arthur river stock is a reproductively active population capable of self-recruitment or a marginal satellite population of epizootic recruits spawned from a western Victorian estuary (Shaddick et al. 2011). Due to the restricted distribution and data deficiency of estuary perch in Tasmania, the Inland Fisheries Service (IFS) has not listed the conservation status of the species and currently regulates the fishery with a catch and release protocol.

1.2 *Age-truncation and growth*

Like most percichthyids, estuary perch are long-lived and slow-growing (up to 41 years old) (Walsh et al. 2010). The advantage of longevity is that populations can survive long periods

of unfavourable reproductive conditions such as droughts and floods without a population collapse occurring (Longhurst, 2002). However, the disadvantage of longevity is that excessive fishing pressure can cause age truncation and reduce the reproductive potential of the stock (Longhurst, 2002, Beamish et al. 2006, Walsh et al. 2010). The combined effects of estuary perch's morphology, catadromous migration and spawning aggregation behaviour, makes the species highly susceptible to gillnet capture. In Tasmania, commercial and recreational gillnetting in estuaries was relatively common during the 1950's – 1980's and could have caused estuary perch stocks to become recruitment overfished. Furthermore, estuary perch exhibit episodic recruitment, with age structures displaying strong, weak and missing cohorts (Walsh et al. 2010). Walsh et al. (2010) suggests that stronger age classes in Victorian and NSW estuary perch stocks occur when floods coincide with spawning events. However, the relationship between environmental flows and recruitment variability in estuary perch is poorly understood, as latitudinal effects have marked influences on the timing of spawning (Walsh et al. 2010). The effects of recruitment overfishing and recruitment variability combined with the construction of in stream barriers to fish passage (e.g.: road culverts and weirs) are likely factors that contributed to the localised extinction of Tasmanian estuary perch stocks.

Growth rates of estuary perch vary between sexes with females reaching larger asymptotic lengths, faster than males (Walsh et al. 2010). The maximum reported age of 41 years makes estuary perch the second longest living percichthyid in the world (Anderson et al. 1992). No latitudinal plasticity in growth has been reported for estuary perch however, differences in growth rates have been recorded for the closely related Australian bass (Harris 1987). Assessing spatial variations in growth is important when considering the

vulnerability of populations to various exploitation rates (Gilliers et al. 2006). The Arthur River is on the edge of the southern distribution for estuary perch and defining age classes for this population could provide insight into how population growth rates are affected by cooler climates. Plasticity in growth with latitudinal shifts is not uncommon for Australian estuarine species and exposing such differences could play a vital role in management of the species (Phelan et al. 2000, Sarre & Potter 2000, Stocks et al. 2011).

Age estimates are primarily based on enumerating periodic increments on hard structures such as otoliths, scales, vertebrae and fin spines (Campana 2005, Seo et al. 2006, Bubley et al. 2012, Luque et al. 2014). Otolith analysis produces the most accurate age estimates and can also be used to model growth (Stocks et al. 2011). Growth in early life stages is an important factor to consider when determining the future success of stocks. By using back-calculations of otolith increments, historical growth of individual fish can be correlated with annual environmental changes (Thresher et al. 2007, Black et al. 2011). Additionally, various data points can be drawn from a single otolith including length estimates for young fish that are not selected by fishing methods (Morita & Matsuishi 2001, Ballagh et al. 2006).

1.3 Reproduction and spawning

Determining the spawning behaviour and reproductive biology of a species is essential to effectively manage the fishery (King 1995, Sponaugle et al. 2002). Spawning sites, seasons, relative fecundity and sizes at maturity make up some of the critical baseline data used for stock assessments (King 1995). Despite the economic and recreational value of estuary perch in Australia, this information is lacking in Tasmania.

In mainland rivers, males mature early at a fork-length (FL) of 22cm while females mature later at a FL of 25cm (Walsh et al. 2011). This is consistent with other species of percichthyids (Harris 1986, Cazorla and Sidorkewicz 2008). The optimal salinity for larval survival for Australian bass is 25-35‰ and temperatures of 18°C and estuary perch are believed to spawn in similar conditions (Trnski et al. 2005). Estuary perch are broadcast spawners that select structurally complex wooden debris for spawning (van der Meulen et al. 2014). Estuary perch spawn during winter in NSW and as late as spring in Victorian rivers (Walsh et al. 2011). The spawning period is yet to be determined in Tasmania, and is crucial for future management of the species.

Recruitment rates of estuary perch are dependent on river flows during spawning periods as flooding may delay, prevent or promote spawning (McCarragher and McKenzie 1986, Walsh et al. 2011). To compensate for episodic recruitment, estuary perch have a high batch fecundity which is significantly increased in older fish (Walsh et al. 2011). Fecundity rates can vary between populations of percichthyids (Bath and O'Connor 1982) and estimates have been inconsistent between authors for estuary perch (McCarragher and McKenzie 1986, Walsh et al. 2011). Estuary perch are believed to be group synchronous spawners because of differences in oocyte development, suggesting individual fish can spawn multiple times during a breeding season (Walsh et al. 2011). However, the number of times an individual female can spawn in one breeding season remains unknown. There is also uncertainty regarding sex ratios during the broadcast spawning events (Walsh et al. 2011).

1.4 *The Arthur River estuary*

The Arthur River estuary is located on the Northwest coast of Tasmania (41°02'56"S, 144°40'09"E). The river is one of the most wild and untouched waterways in Tasmania with no record of logging or damming. The estuary is large (exceeding 14km) and the upper freshwater component spreads into a network of tributaries, reaching 180 km in length (Beard et al. 2008).

The Arthur River area has been listed as of high conservation significance (class B) and supports a diverse range of bird and aquatic life, many of which are of conservation importance (Beard et al. 2008, DIPIPWE 2014 and Edgar et al. 1999). Estuary perch have also been reported in abundant numbers, making up 20% of fish catches in the lower estuary (DPIPWE 2014).

In the lower areas of the estuary, the river banks are steep and often covered in complex wooden structures. These structures can extend from the fresh water surface layer to the benthic saline waters throughout the estuary, indicating there is an abundant amount of spawning habitat for estuary perch (Walsh et al. 2014). However, parameters such as salinity and dissolved oxygen (DO) have been known to fluctuate after rainfall events and may impact spawning opportunities and larval survivability of estuary perch.

1.5 *Estuary perch potential in Tasmania*

Research shows that participation in recreational fishing in Tasmania is high, and the industry has an important economic benefit for the state. (Yamazaki et al. 2013). Estuary perch is recognized as one of the key species targeted by recreational fishers in Victoria

(Kemp et al. 2012) suggesting that similar success could be achieved with this species in Tasmania, if they occurred in abundant numbers. In Victoria, the Department of Environment and Primary Industries (DEPI) implements a stocking program in both fresh and marine environments to enhance their estuary perch fisheries (DEPI 2014, Torrens et al. 2002). The Tasmanian IFS could follow in the footsteps of DEPI Victoria and utilise the species through a breeding program to enhance recreational fishing with the additional benefit of improving the conservation status of estuary perch in Tasmania.

The option of purchasing and importing estuary perch fry from Victorian hatcheries, to re-establish Tasmania's fisheries presents an attractive economic prospect, however this may be hampered by biological and genetic dissimilarities. If the Arthur river stock is genetically dissimilar to the western Victorian stock, the establishment of a hatchery and re-stocking program within Tasmania using local brood-stock sourced from Arthur River would be required. However, no research has been done to assess the status, age structure, growth profile or reproductive capacity of the remaining Tasmanian stock. Without a proper understanding of the population dynamics and status of estuary perch in the Arthur River, future conservation and management efforts must rely on extrapolated information from mainland research.

Biological, genetic and population dynamic research on estuary perch is needed in Tasmania as this information forms the basis for stock assessments and can provide the foundation for future monitoring and research to restore stocks. Prior to site selection for restocking, it is crucial to understand environmental constraints on growth to optimise both species survival and species fitness, particularly for a recreational fishery. Additionally, an understanding of

biological parameters is required to plan and predict the likelihood of a successful environmentally friendly establishment.

Over the past 5 years estuary perch have become a popular target for recreational sport fishing enthusiasts in Tasmania. As more fishers are discovering the potential of this percichthyid, on-going pressure is placed on management authorities to enable recreational fishers to utilise this resource. This study is critical to provide information to management authorities in order meet the demands of recreational fishers and ensure the continued sustainability of estuary perch in Tasmania.

1.6 Project aims

Primary aim

To determine key biological population dynamic parameters for the Arthur River estuary perch stock.

Specifically

1. To obtain preliminary estimates of stock size (based on mark-recapture methods).
2. To determine size and age stock structure of estuary perch and develop growth models.
3. To determine the suitability of using scales or anal fin spines as a non-lethal aging structure for estuary perch.
4. To investigate the reproductive capacity of the estuary perch stock in the Arthur River (if possible, determine size and age of maturity and fecundity relationships with size and age).
5. To provide advice for future management options for the species in Tasmania to the IFS.
- 6.

2.0 Materials and methods

2.1 *Study region and sample selection*

Estuary perch were collected in the Arthur River (41°02'56"S, 144°40'09"E) (Figure 1) between December 2014 and August 2015. Estuary perch were caught using monofilament gillnets with varying mesh sizes and on rod and line. A hydrolab (version DS5) was used to measure salinity and temperature at depth from sites (Figure 1) located at the mouth to the end of the salt wedge in 500 - 1000m intervals along the Arthur River. This data was used in conjunction with GPS and biological catch data to identify spawning areas for estuary perch. Fish were collected in summer, autumn and winter to determine biological changes for estuary perch in the Arthur River. Due to the uncertain conservation status of estuary perch in Tasmania, permits were granted, authorising the euthanasia of 90 fish (Animal Ethics Permit No A14546 and IFS Permit No 2014-51). Excess captures were tagged and released to monitor the stock during the study and to provide population estimates for the IFS.

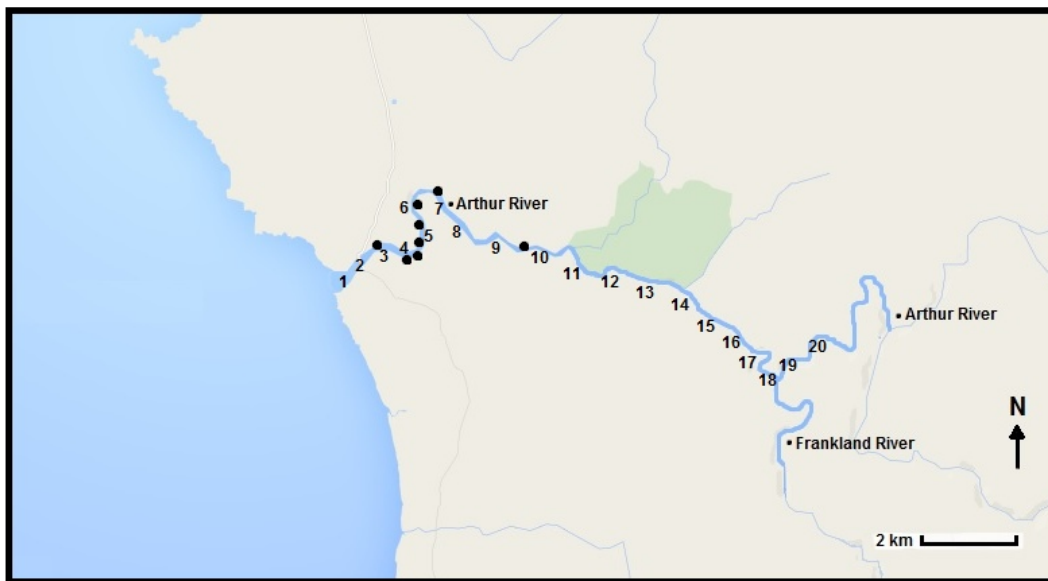
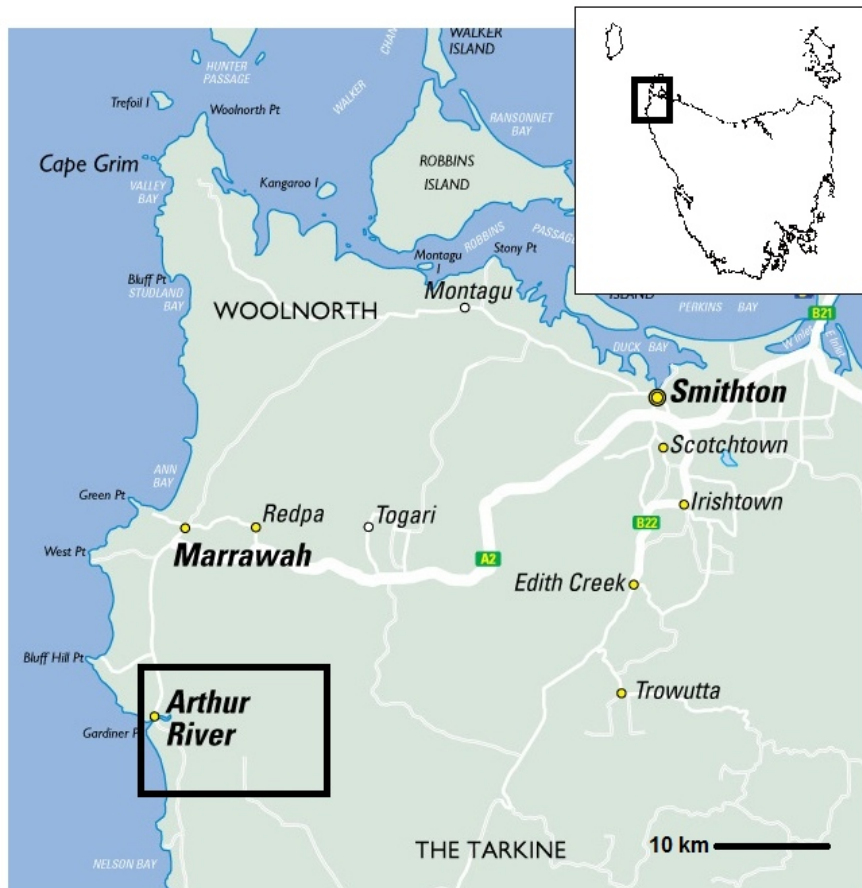


Figure 1. Study region and site selection on the Arthur River, Tasmania. Black dots represent estuary perch (*Percalates colonorum*) capture sites. Values indicate sites where water quality parameters were obtained.

2.2 Processing – euthanised fish

Fish selected for euthanasia were placed into a bath containing clove oil (2ml per 30 litres) to cause death by overdose, prior to being placed on ice for later dissection and sample collection. During dissections, FL (± 1 mm) and total weight (± 1 g) were recorded, and samples of scales (3-6 from behind the pectoral fin), muscle, caudal fin clips and anal fin spines collected. Sex was assigned externally by examining the urogenital opening structures near the anus (Figure 2), and subsequently verified by macroscopic examination of the gonads to establish a non-destructive sexing technique. Whole gonads were removed, staged (Table 1) and weighed (± 0.1 g). In females, an anterior sub section of the ovary was taken and weighed (± 0.1 g) before being placed in 70% ethanol for later fecundity estimation and oocyte measurements. Pairs of sagittal otoliths were removed, cleaned and stored to dry in paper envelopes for later age determinations and increment measurements.

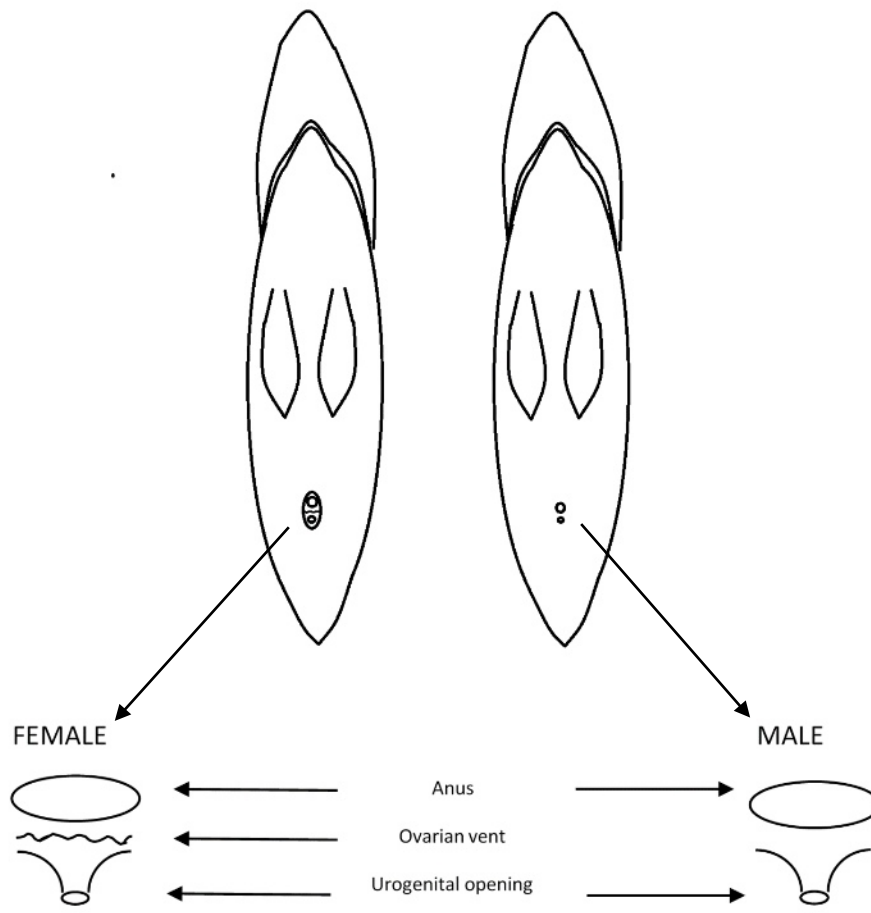


Figure 2. Diagrammatic representation of the external structure of the urogenital opening of estuary perch (*Percalates colonorum*).

Table 1. Macroscopic characteristics and stages of female and male estuary perch (*Percalates colonorum*) adapted from Walsh et al. (2011).

Males	
Stage	Macroscopic characteristics
1. Indeterminate	Small, moderately translucent to pink, determination of sex is difficult.
2. Developing	Much larger than stage 1, colourless to white, transverse sections triangular.
3. Mature	Testes larger in diameter, white in colour, slightly vascularised
4. Ripe	Swollen, soft and white testes. Milt flows from the urogenital pore.
5. Spent	Rubbery, reduced in size, bloodshot and grey in appearance.
Females	
Stage	Macroscopic characteristics
1. Indeterminate	Small, moderately translucent to pink, determination of sex is difficult.
2. Developing	Much larger than stage 1, colourless to cream with fine granular texture. transverse sections circular. Slightly vascularised.
3. Mature	Ovaries larger in diameter, yellow in colour with extensive vascularisation. Oocytes visible.
4. Ripe	Hydrated translucent oocytes visible through ovary wall, yellow to amber in colour, gonads take up three quarters of body cavity.
5. Ovulation	Oocytes extruded from the genital papilla with gentle gravitational pressure.
6. Spent	Ovaries reduced in length, leathery, bloodshot towards posterior end.

2.3 Processing – tag and release fish

Excess fish not euthanised at capture were placed in oxygenated holding tanks prior to being tagged and released. During tagging procedures, individual fish were removed from the holding tank, measured (fork length ± 1 mm), sexed (by external inspection), double tagged with T bar tags among fin ray supports, and samples of scales taken. Small immature fish that were difficult to sex were classed as indeterminate (I). Once fish were tagged they were released back into the river.

2.4 Laboratory processing

2.4.1 Fecundity and egg size estimates

Sub-samples of ovarian tissue stored in 70% ethanol were removed, placed on paper towelling to blot dry for 1 minute and re-weighed (± 0.1 g) to calculate a correction factor (CF) for dehydrating weight losses whilst being stored in ethanol. Once weighed, a small piece of ovarian tissue was cut from each sub-sample and weighed (± 0.1 g) before three random sections (approx. 0.01 g) were cut and weighed to an accuracy of 0.0001 g. These sections were placed in a vial of saline water and vigorously shaken to separate oocytes from connective tissue. The separated oocyte mixture was poured into a petri dish and rinsed to ensure all oocytes were transferred. Filamentous connective tissue not containing oocytes was removed using tweezers under a dissection microscope. The petri dish was swirled clock wise to draw oocytes away from the edges and into the centre to make counting easier. Oocytes were viewed with a dissection microscope and photographed. Oocytes were automatically counted and measured using image-J version 1.38 (see Appendix A for full method). Hydrated oocytes in estuary perch vary from 0.6mm – 1.2mm

in diameter (Walsh et al. 2011). Only oocytes in this size range were used to calculate batch fecundity. Total ovarian fecundity was based on the total oocyte count, regardless of individual oocyte size. Oocyte counts were divided by their respective subsample weight and averaged across the triplicates to determine the number of eggs per gram of ovarian tissue in each individual. Fecundity estimates were estimated gravimetrically where the mean number of eggs per gram of fixed tissue was corrected for weight losses from fixing process prior to being multiplied by the total wet weight of the gonads. The number of potential batches produced in a single spawning season by each individual was calculated by dividing ovarian fecundity by batch fecundity (Zamarro 1991).

2.4.2 Age determination

Otoliths

Otoliths were individually weighed (± 0.0001 g), embedded in clear casting resin and transverse sections of approximately 300 μ m were cut using a Gemmasta multi-cut diamond saw. Cut sections were polished with a 1200 grit faceting wheel and mounted on labelled glass slides. Slides were viewed under a dissecting microscope with reflected white light against a black background. Ages were determined by counting opaque zones along the dorsal edge of the sulcus (Figure 3).

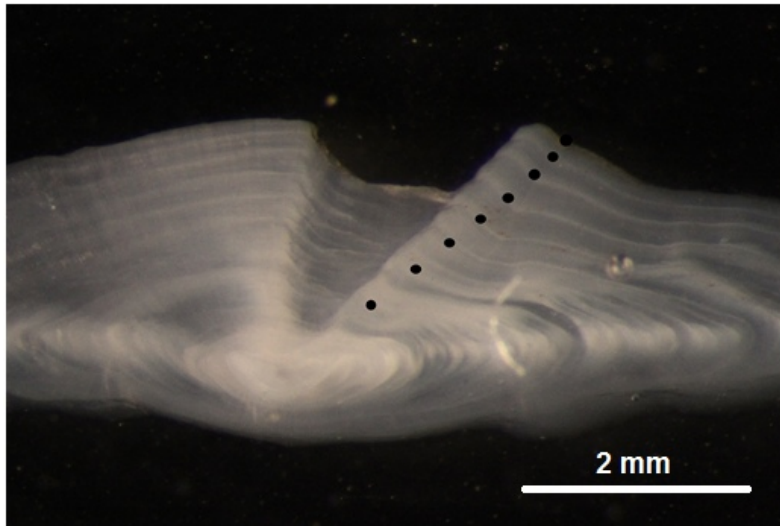


Figure 3. Sectioned estuary perch (*Percalates colonorum*) otolith (aged 8). Black dots indicate opaque zones.

Scales

Scales were cleaned and dried in the laboratory before being examined under white light for signs of scale loss. Lost scales were discarded while birth scales were mounted between two glass slides for microscopic examination. Scales were viewed with a dissecting microscope with white light on a black background. Ages were determined by counting the number of circuli from the primordia to the edge on the concaved side of the scale. (Figure 4).

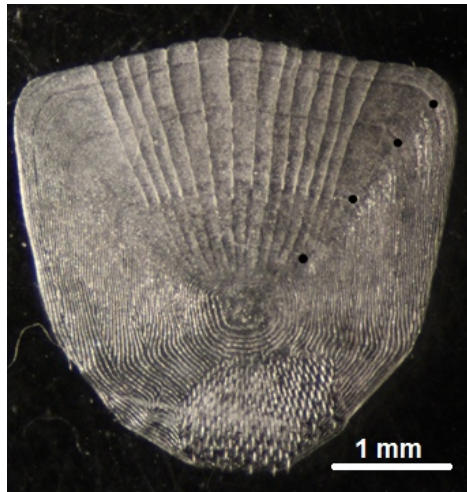


Figure 4. Mounted estuary perch (*Percalates colonorum*) scale (aged 4). Black dots indicate circuli used for age estimates.

Spines

Anal spines were boiled and cleaned before being embedded in clear casting resin. Once cured, sections of approximately 300 μ m were taken, polished and mounted on glass slides. Ages were determined by counting annuli using the same techniques as Sullivan (1977) and Metcalf and Swearer (2005) (Figure 1).

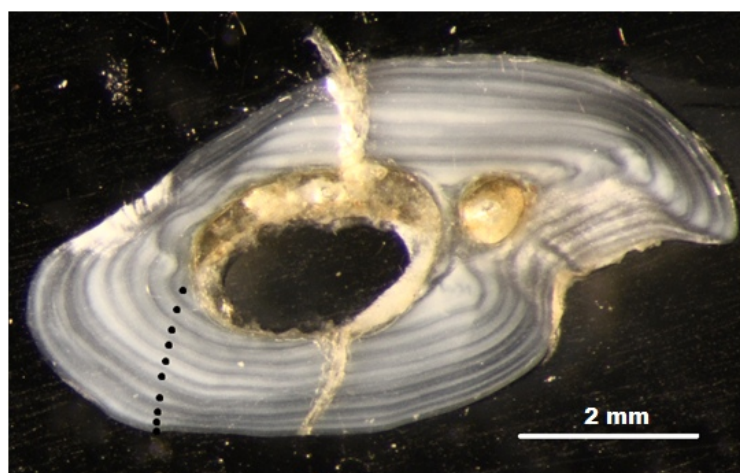


Figure 5. Sectioned estuary perch (*Percalates colonorum*) anal fin spine (aged 10). Black dots indicate growth rings used for ageing.

All otoliths, scales, and spines were read three times without knowledge of fish size, sex or previous estimates. All hard parts were scored by their readability and ranked accordingly (Table 1). The precision of age estimates were calculated using the index of average percent error (IAPE) for each structure (Beamish and Fournier, 1981).

Equation 1. Index of average percent error equation

$$IAPE = \frac{1}{N} \sum \left(\sum_{i=1}^R \frac{(X_{ij} - X_j)}{X_j} \right) \times 100$$

Where

N is the number of fish aged

R is the number of times the fish are aged

X_{ij} is the i^{th} age determined for the j^{th} fish

X_j is the mean age determined for the j^{th} fish

All unreadable sections (5) were excluded in this study (Figure 6).

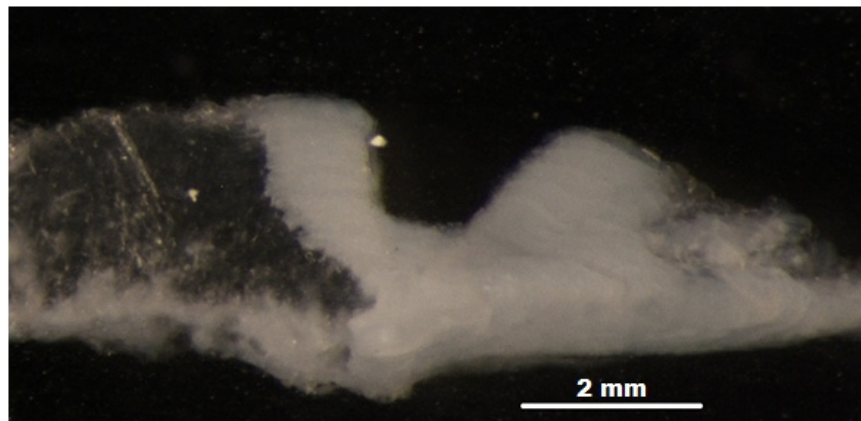


Figure 6. Example of an unreadable estuary perch (*Percalates colonorum*) otolith with a readability score of 5.

Table 2. Otolith, scale and anal fin spine readability scores adapted and modified from Vigliola and Meekan (2009).

Readability Score	Reader Interpretation
1	Increment exceptionally clear and easy to read
2	Section is unambiguous but not as clear as score 1
3	Section is subject to two interpretations
4	Section is subject to multiple interpretations
5	Section cannot be read

2.4.3 Back-calculation

Only scales and otoliths were used for back calculations as a hard part origin could not be assigned in fin spines. For scales, older fish with age deviations of more than 3 years compared to otoliths were not included in back calculations. Photographs were taken of sectioned otoliths and mounted scales with a Cannon 600d Digital SLR camera fitted to a dissection microscope. Microscopic grids were also photographed to apply as a scale in image J for increment measurements and back calculations.

Transects were set digitally from the primordia of otoliths and scales to the outer edges of ventral proximal sides using image J analysis software (Figure 7). Increments were marked using the plugin 'Object J'. Distances between increments were measured and calculated into percentages of the transect length (radius). These percentages were directly applied to individual growth and used to predict FLs at previous ages by multiplying cumulative proportions by the size at capture (Francis 1990).

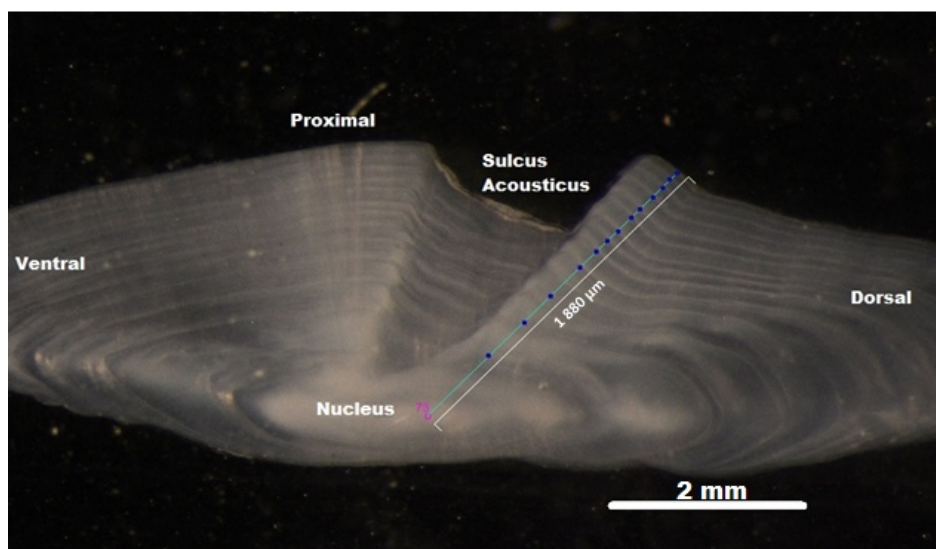


Figure 7. Back-calculation transect of a sectioned 13 year old estuary perch (*Percalates colonorum*) otolith.

2.5 Growth analysis

2.5.1 Von Bertalanffy growth curves

Growth was modelled using the von Bertalanffy growth equation (equation 2). The parameters L_{∞} , K and t_0 were produced from size at age data and the model was fitted using ordinary least squares regression analysis (King 1999).

Equation 2. Von-Bertalanffy grow equation

$$L_t = L_{\infty} (1 - \exp(-K(t-t_0)))$$

Where

L_t is length

L_{∞} is the asymptotic length where growth is zero

K is the growth rate

t is time

t_0 is the theoretical age at zero length

Multiple Von Bertalanffy growth curves were developed for female and male estuary perch from scales and otoliths using raw lengths, back calculated lengths and constraining t_0 to 0. Models were also produced where ages were constrained between 5 and 15 years to avoid young and old fish from skewing data.

2.6 *Mark-Recapture and population estimates*

Eighty five estuary perch were tagged and released during two fishing trips; 17 tagged in December (2014) and 68 tagged in early January (2015). Two sampling trips took place; one in March and one in August. Recaptured fish were recorded on both trips and two population estimates were derived from the Peterson mark-recapture equation (equation 3) before a final population estimate was determined. Confidence intervals (95%) were calculated by multiplying standard error by 1.96. (King, 1995).

Equation 3. Peterson mark-recapture method and standard error calculation

$$\frac{T}{N} = \frac{R}{C}$$

$$SE = \sqrt{\left[\frac{T \cdot C \cdot (C - R)}{R^3} \right]}$$

Where

T = Number of tagged fish

R = Recaptured fish

C = Total number of caught fish (tagged + untagged)

N = Number of fish in the population

2.7 Analysis

Regression analysis and correlations were used for relationships between fecundity, size and age; otolith age, scale age and fin spine age; length, otolith and scale radius.

A one-way ANOVA was used to differentiate mean ages for otoliths, scales and anal fin spines. Significant differences were explored with a post hoc pairwise comparison using a Tukey honestly significance difference (HSD) test.

A Two-way ANOVA was used to test differences in mean readability scores for fish aged <8 years and >9 years between different hard part ageing structures. A Tukey HSD post hoc was used to uncover where the differences occurred.

Comparisons of growth curves were made between sexes of estuary perch for raw and back-calculated length-at-age data using scales and otoliths. Comparisons were also made constraining t_0 to 0 and for fish aged between 5 and 15 years old. All comparisons were tested for significance ($\alpha = 0.05$) using the analysis of residual sum of squares (AoRSS) (Haddon 2011).

3.0 Results

3.1 Sample description

A total of 189 estuary perch were caught in the Arthur River, within this sample, 89 fish (68%) were euthanised for biological processing. Although the female to male sex ratio was relatively even (1:1.2) and lengths ranged from 20 to 43cm for both sexes, the length frequency distributions displayed marked multimodal differences. Females dominated the

larger size classes >34cm while males dominated smaller size classes between 20 – 33cm (Figure 8).

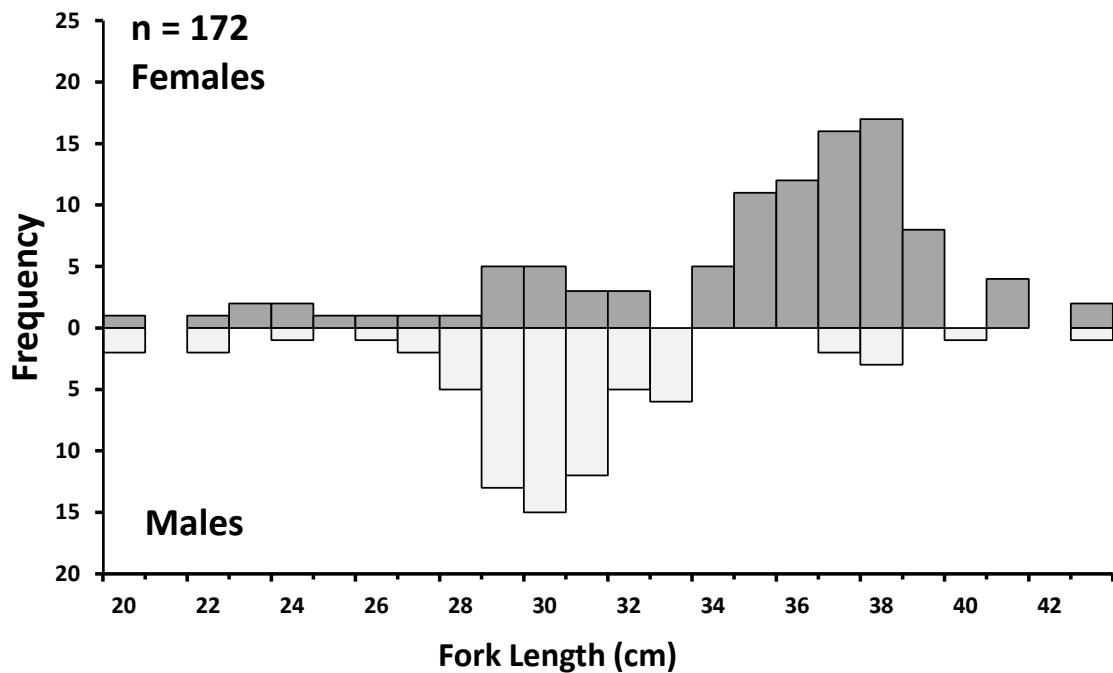


Figure 8. Length frequency distribution of estuary perch (*Percalates colonorum*) caught in the Arthur River, Tasmania. Samples include tagged and euthanised specimens.

3.2 Population estimate

Eighty five estuary perch were caught (44 males and 41 females) from spawning aggregations, tagged and released during two trips within the Arthur River estuary; 17 fish in December (2014) and 68 fish in January (2015). In January, 2 fish were recaptured however were not used in population estimates due to the likely violation of the dispersal Peterson mark-recapture assumption (Cowley and Whitfield 2001).

March survey:

$$N = \frac{C(37) * T(85)}{R(2)}$$

$$N = 1,573 \pm 1081$$

August survey: $N = \frac{C(38) * T(85)}{R(2)}$

$$N = 1,615 \pm 1112$$

A final population estimate of 1,594 (± 775) estuary perch susceptible to fishing gear was obtained with a recapture rate of 4.7% in the Arthur River.

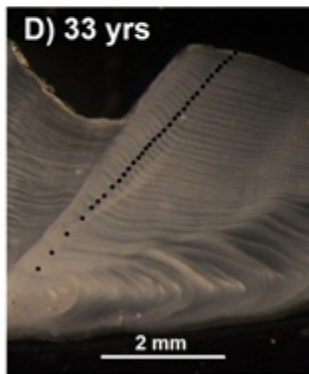
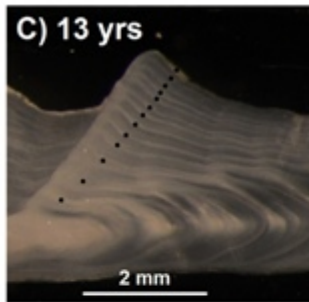
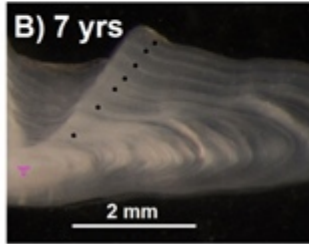
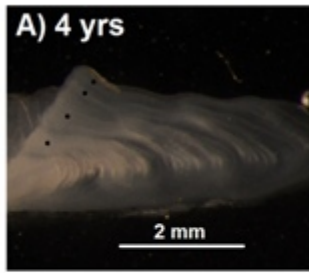
3.3 *Hard part interpretation and readability*

All sectioned otoliths displayed clear continuous translucent and opaque zones (Figure 9A – D). Cores consisted of dense opaque formation and were easily identified. False opaque zones were found within the translucent regions of the first and second banding however were easily identified due to their lack of continuity. Otolith estimated ages displayed the lowest IAPE of all hard parts at 2.3%.

Most sectioned anal fin spines showed distinguishable translucent and opaque banding (Figure 9Ai - Di). False opaque zone formation was more common than otoliths, inconsistent among ages and often hard to distinguish. Reabsorption of inner bands occurred in the vascularised areas (hollow parts) of older fish however no trends could be developed to adjust ages and compensate for this problem. Anal fin spine estimated ages had an IAPE of 8.6% which was the highest among the hard parts.

Scales exhibited circuli radiating from the central core with regular repeatable patterns of growth checks (convergent banding) extending to the outer edge (Figure 9Aii – Dii). False checks were frequent in older fish and were often hard to distinguish from annuli. In younger fish (<8 years), checks were easily identified and often clearer than otoliths. Annual checks in older fish (9> years) were regularly fused together towards the edge and were hard to differentiate between one another. The IAPE for scale estimated ages was 6.1%.

Terminal



Non-destructive

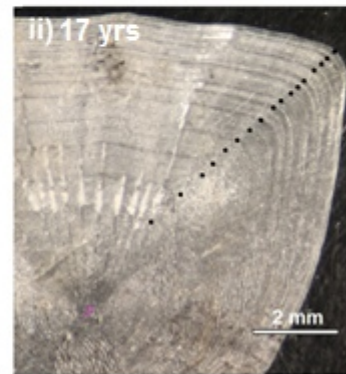
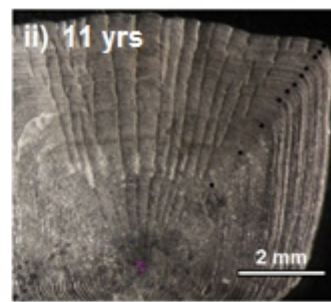
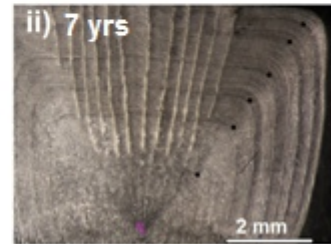
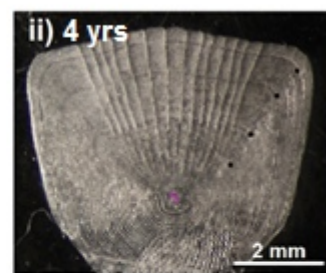
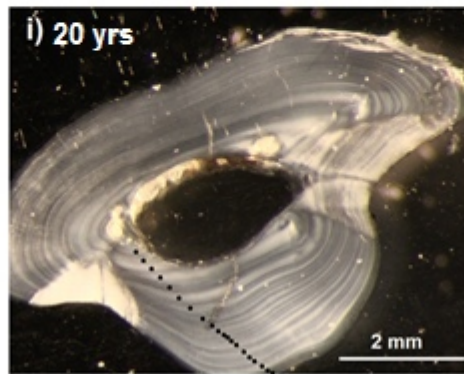
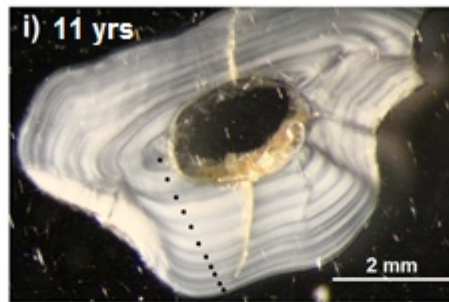
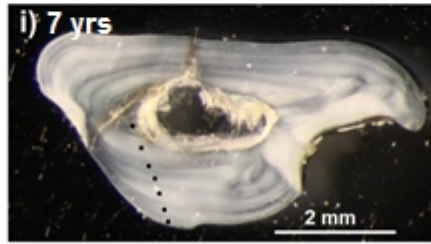
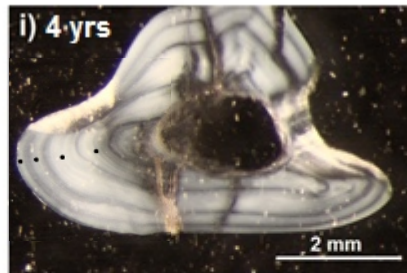


Figure 9. Estuary perch (*Perca latipes*) sectioned otoliths, anal fin spines and mounted scales from individuals aged 4, 7, 13 and 33 yrs. Ages indicate annual counts obtained for each hard part. Images were taken with a dissection microscope with reflected white light on a black background.

There was no significant differences between the readability of hard parts and sex for estuary perch ($p = 0.8$). Consequently, data was pooled to look for differences between hard part readability with age. Due to violations in ANOVA assumptions, significant differences could not be tested across all age classes (). Therefore, additional pooling was used to separate ages into young (4 – 8 year olds) and old (9+ year old fish) categories.

There was a significant difference in the readability of otoliths, scales and anal fin spines for young and old estuary perch (ANOVA, $F=17.71$, $df = 2$, $P<0.001$). Otoliths displayed low readability scores overall, and did not vary with different ages for estuary perch. The readability scores for scales were low for young fish but consistently became worse with increasing ages (and 11). Younger fish aged between 4 and 8 years old had the lowest readability scores of all hard parts however, older fish (>9 years) were the hardest to read. Anal fin spines displayed moderate readability scores throughout all ages with slightly higher scores for older fish (Figure 11).

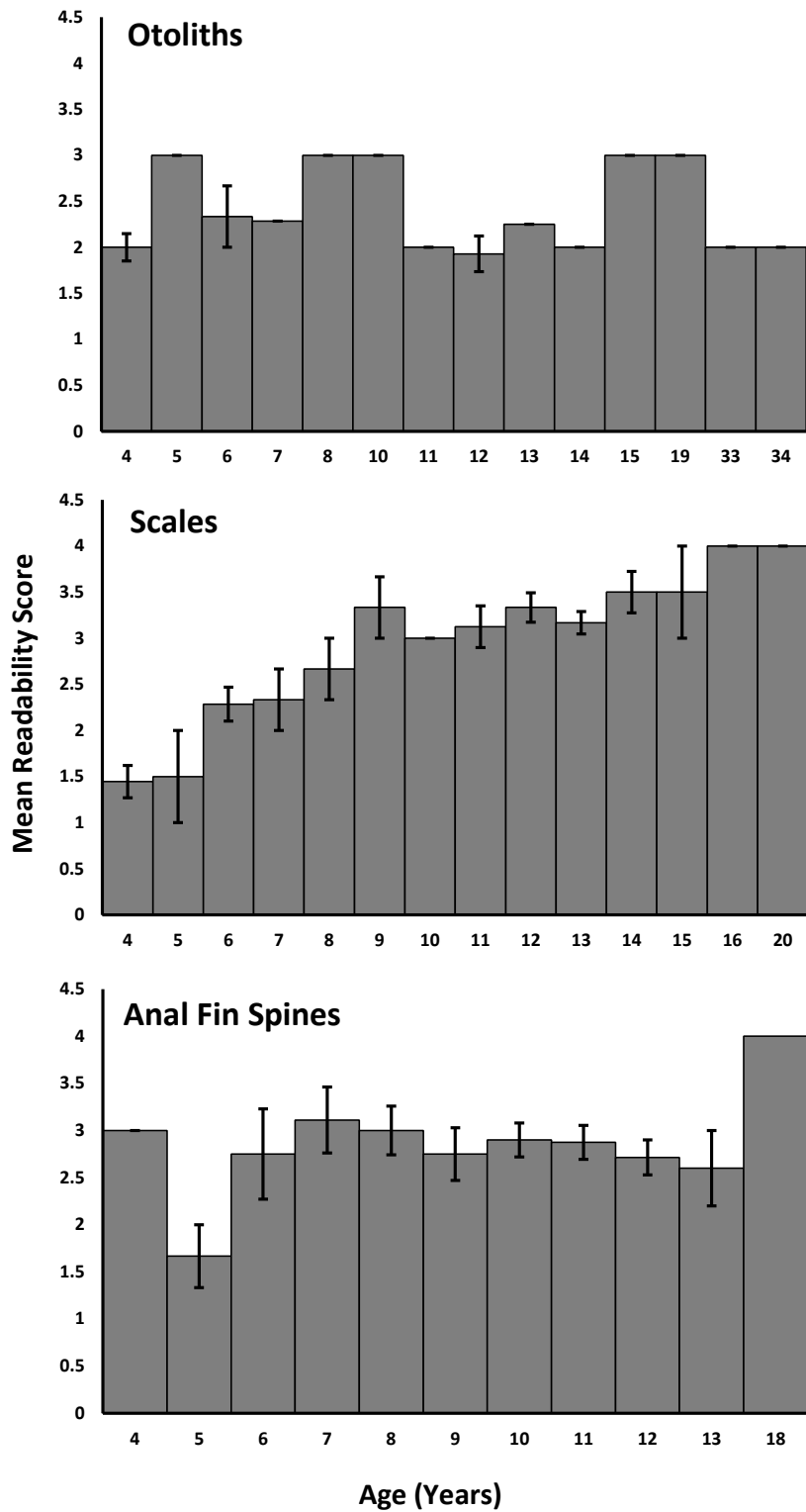


Figure 10. Mean readability scores (\pm SE) of individual age classes for estuary perch otoliths, scales and anal fin spines.

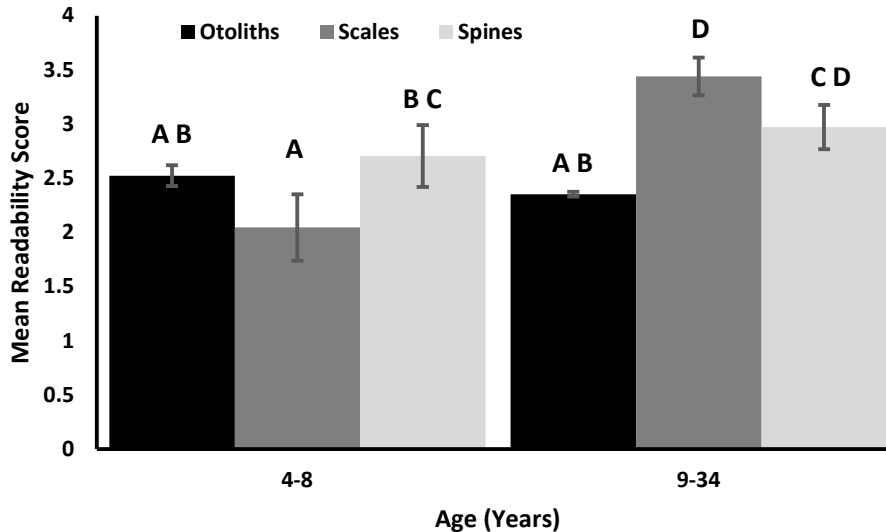


Figure 11. Mean readability scores (\pm SE) of young (4 – 8 year olds) and old (9-34 year olds) estuary perch otoliths, scales and anal fin spines. Letters indicate significant differences between groups.

3.4 Age estimates of hard parts

Overall, age estimates derived from scales and anal fin spines did not coincide with age estimates from otoliths for females and males (Figure 12). There was a significant difference between the estimated ages of all hard parts (*ANOVA*, $F = 5.76$, $df = 2$, $P = 0.004$). A Tukey HSD post-hoc outlined that both fin spines and scales underestimated the ages of estuary perch.

Scales were relatively reliable for younger fish <8 years however over estimated age by up to 2 years and underestimated age by up to 18 years in older fish (Figure 12). Age estimates from anal fin spines were less reliable than scales, even in younger fish and were over and under estimated more consistently than scales. There was a significant correlation between fin spine and otolith ages ($P = <0.001$) and scale and otolith ages ($P = <0.001$). Scale and

otolith age estimates displayed a stronger linear relationship ($R^2=0.66$) than fin spine and otolith age estimates ($R^2=0.60$) (Figure 12).

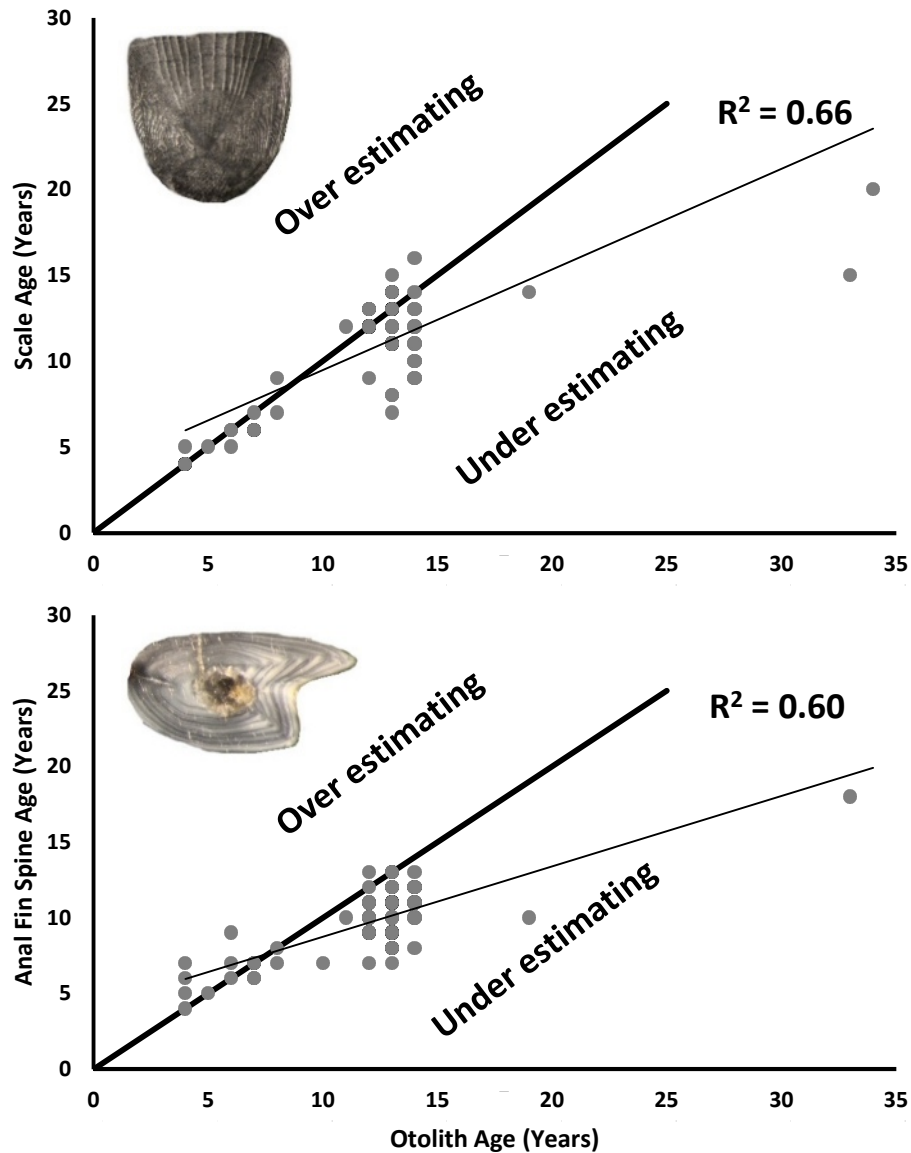


Figure 12. Scatterplot of scale and anal fin spine estimated ages with otolith estimated ages with a line of 1:1 for estuary perch (*Percalates colonorum*) caught in the Arthur River, Tasmania. R^2 values show coefficient of determination under linear regression analysis.

3.5 Age frequency distributions

The maximum age for males was 34 compared to 19 for females however, despite this difference in longevity, the majority of fish (68%) were aged between 12 and 14 years with 13 years being the dominate age cohort for both sexes (Figure 13). A smaller secondary mode of ages between 4 and 8 years was evident in both sexes but fish aged between 9 - 10, 15 – 18 and 20 - 32 were absent in the stock structure.

Age frequencies using scales showed strong and weak year classes to some degree of accuracy for females aged up to 10 years old, but could not identify where missing cohorts occurred. Scale age frequencies showed dangerously false evidence of little variation among male age classes across all size ranges (Figure 13). In contrast, fin spine age frequencies displayed even less variation in cohort strengths among males and females, giving the false impression of relatively stable recruitment (Figure 13).

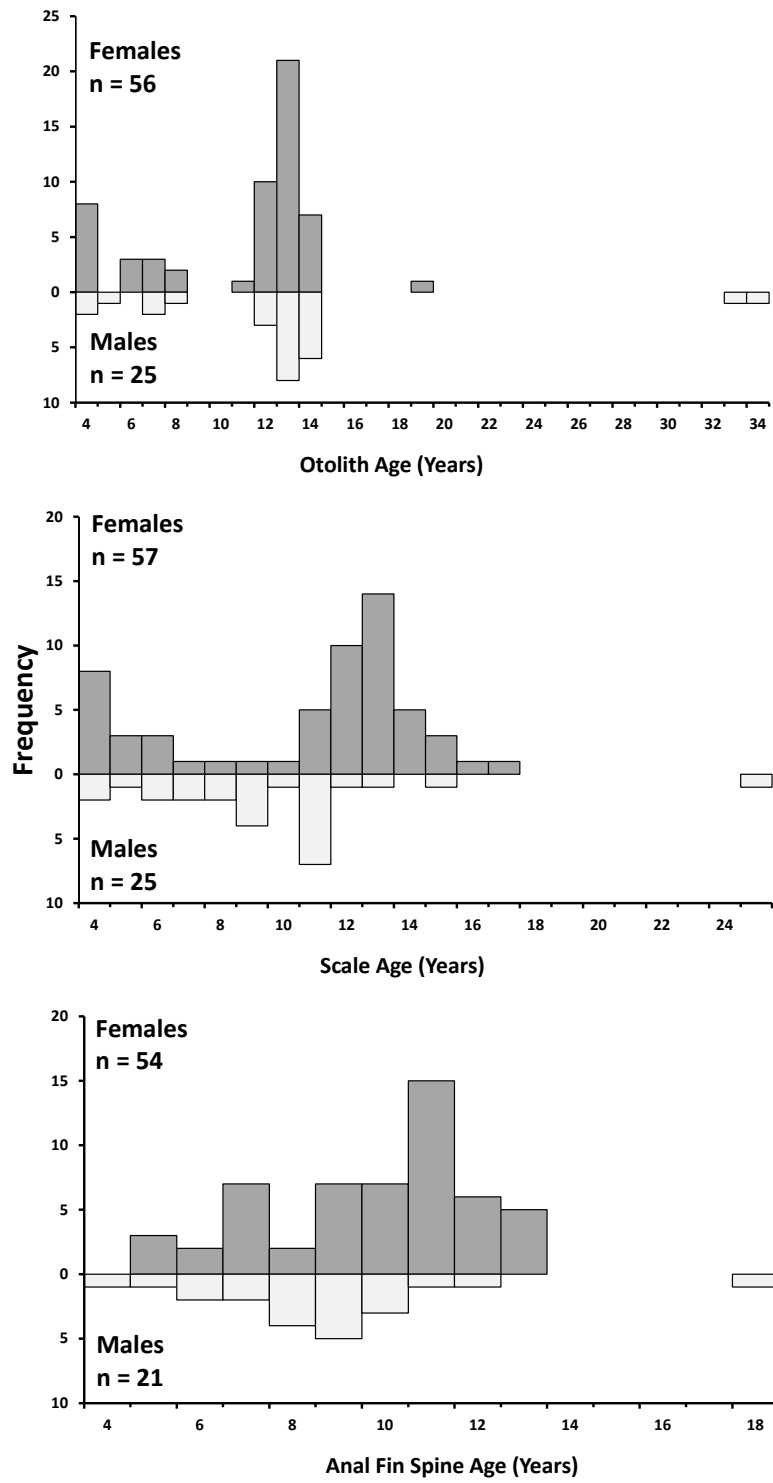


Figure 13. Age frequency distributions using otoliths, scales and anal fin spines for estuary perch (*Perca latipes*) caught in the Arthur River, Tasmania.

3.6 Growth modelling scales vs otoliths

3.6.1 Growth comparisons (sex related differences)

Due to the high degree of inaccuracy, fin spines were excluded from growth models.

Otoliths outlined marked differences in female and male growth. Females obtained higher predicted asymptotic lengths (L_{∞}) and had larger K values (VB growth coefficient) compared to males. Models produced from scales followed similar patterns (Figure 14). Otolith modelling produced an L_{∞} of 45.6 cm for females and 39.7 cm for males. Scales marginally under-predicted asymptotic lengths for females (42.4 cm) but was similar for males (39.3cm).

There was a significant difference between female and male growth ($AoRSS$, $F = 65.78$, $df = 82$, $P < 0.001$). These significant differences were found throughout all models, except for back-calculated scales (constrained to ages 5 – 15) (Appendix B).

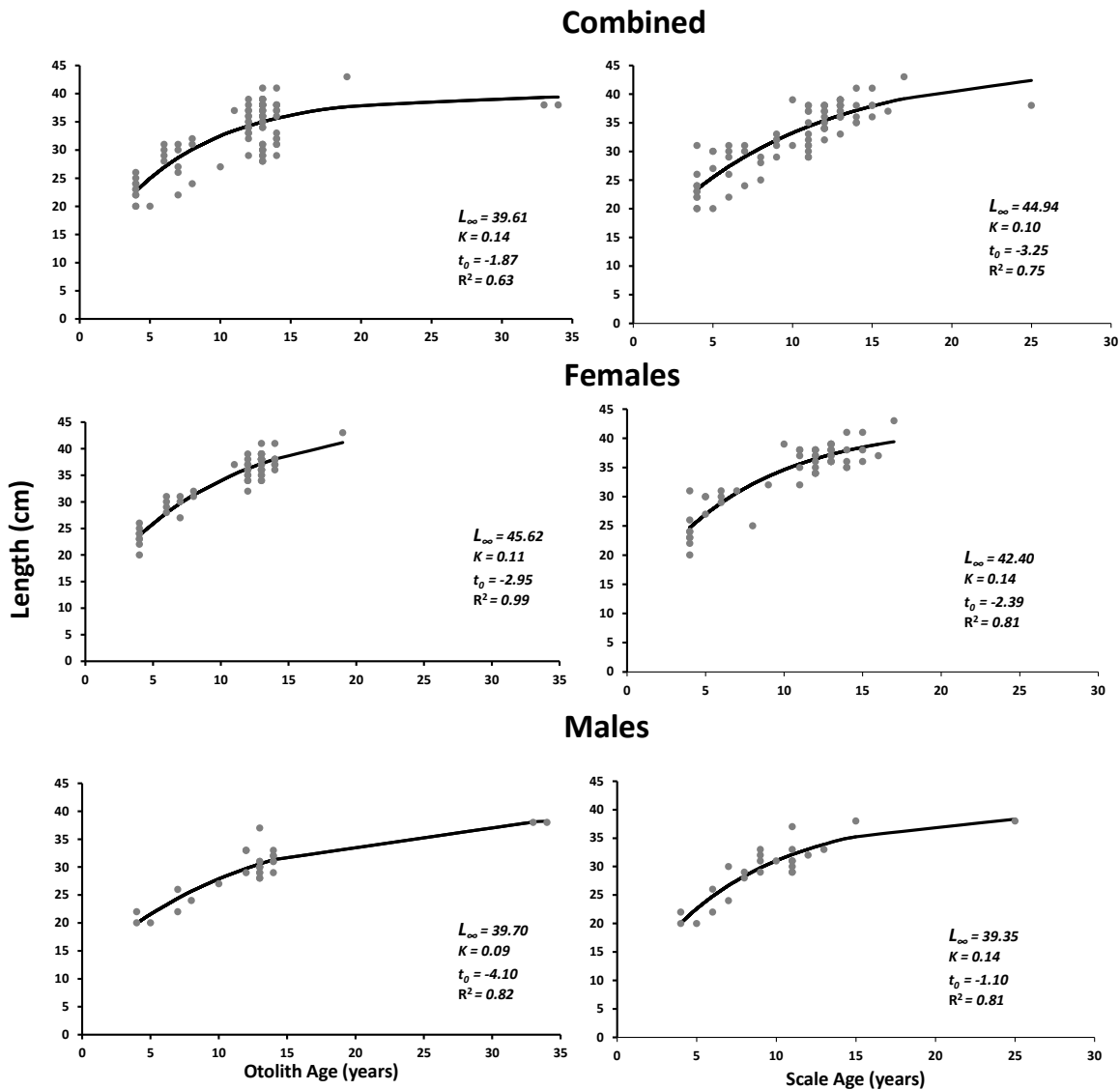


Figure 14. Length-at-age data from otoliths and scales fitted with von Bertalanffy growth curves for male and female estuary perch (*Percalates colonorum*) caught in the Arthur River, Tasmania.

3.6.2 Growth (comparison of models)

There was no significant difference between VB growth parameters (t_0 , K and L_{∞}) generated from models using otoliths and scales for female estuary perch. In contrast, there was a significant difference between growth models generated from scales and otoliths for males (AoRSS, $F = 6.91$, $df = 47$, $p < 0.001$).

3.7 Back-calculated growth

There were significant correlations between otolith (mm) and scale radii (cm) with FL (mm) for estuary perch ($P = <0.001$). As a result, direct proportional back-calculations were applied to generate extensive VB growth models.

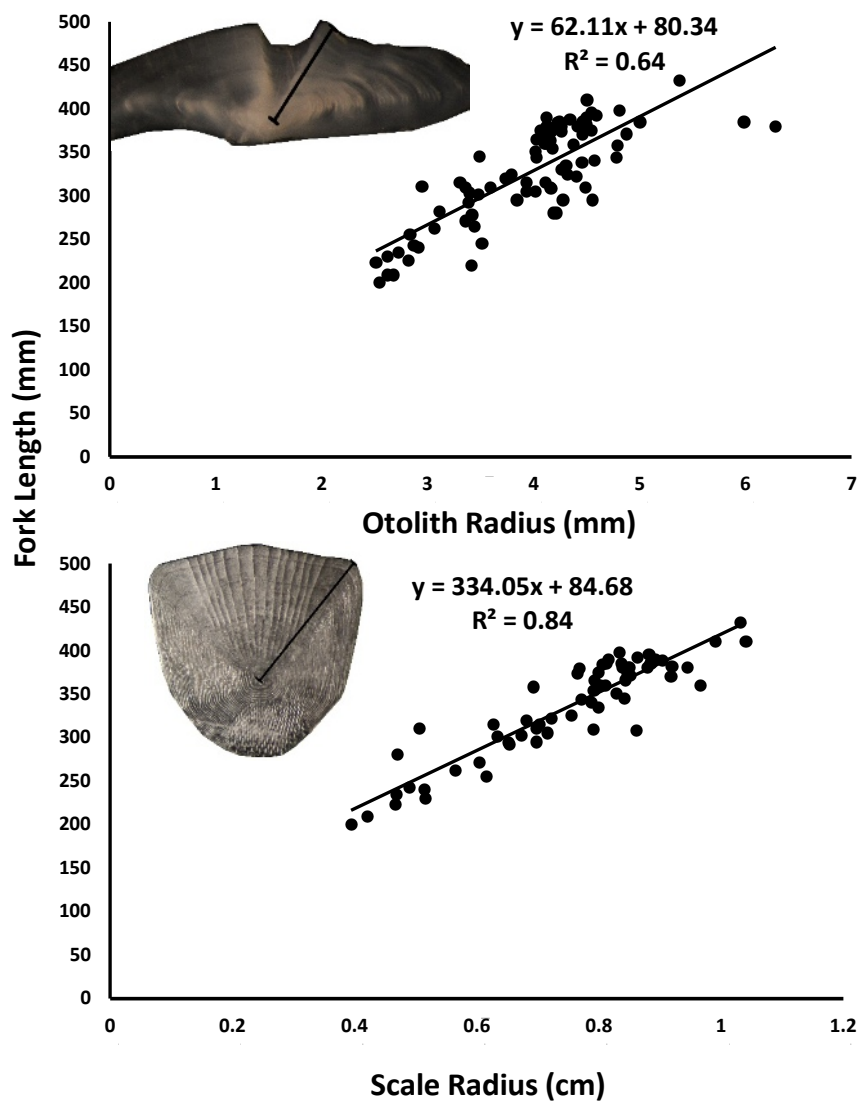


Figure 15. Relationship of otolith (mm) and scale radius (cm) and length (mm) for estuary perch (*Perca latipes*).

Back-calculations (otoliths and scales) produced unreliable growth models and were not accurate enough to expand age-length data. Back calculated models produced inconsistent VB growth parameters when compared with raw scale and otolith generated models (Figure 16). There was a significant difference between otolith back calculated growth models and raw otolith models for females (*ARSS*, $F = 56.63$, $df = 1142$, $P < 0.001$) and males (*ARSS*, $F = 31.81$, $df = 473$, $P < 0.001$). Similarly, scale back calculations also produced significant differences (*females*: *ARSS*, $F = 2.95$, $df = 547$, $P = 0.03$ and *males*: *ARSS*, $F = 5.73$, $df = 367$, $P = 0.01$). There were additional significant differences found across all other comparative models involving back calculated data even with data constraints (Appendix B).

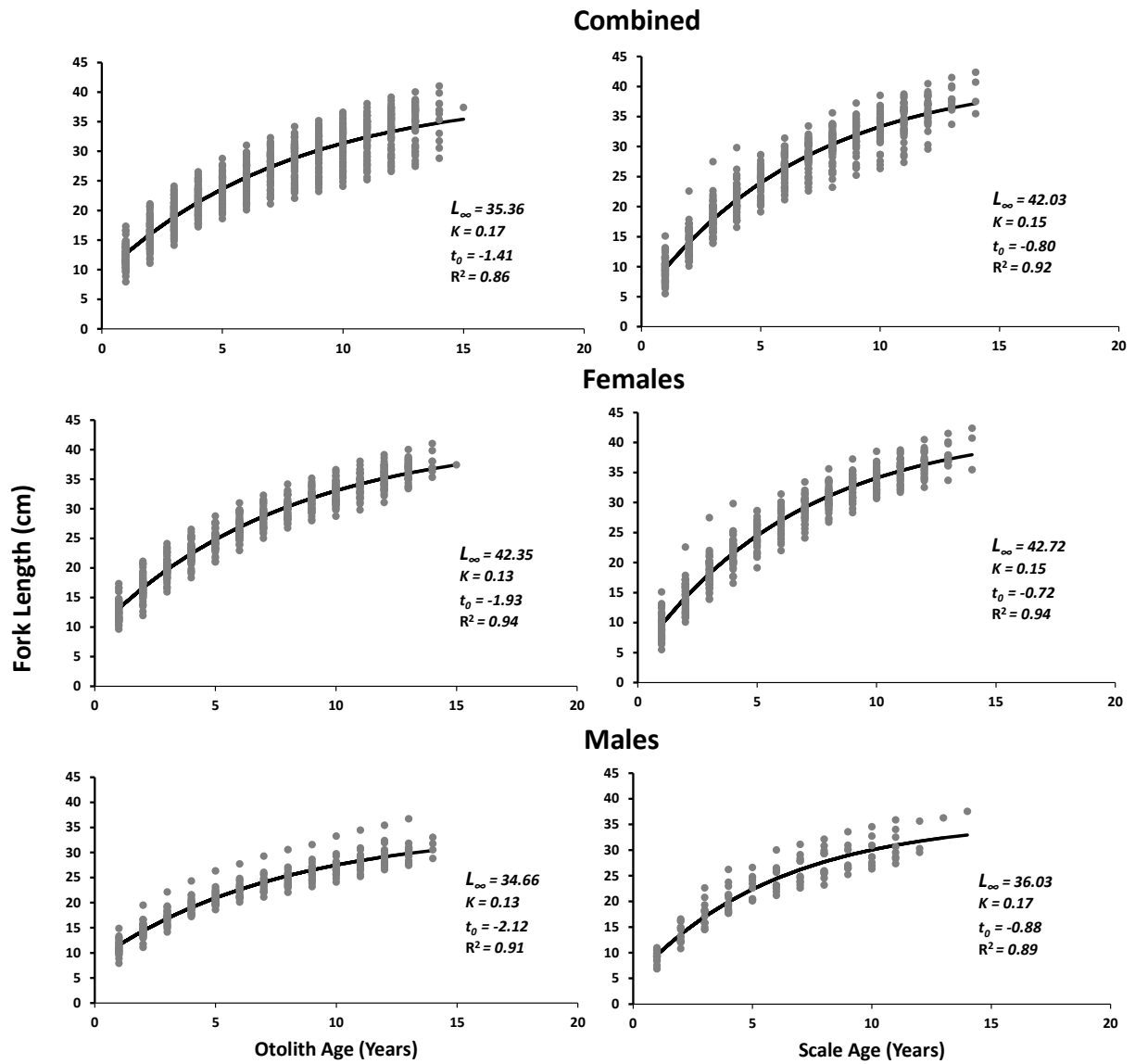


Figure 16. Length-at-age data from back-calculated otolith and scale data fitted with von Bertalanffy growth curves for male and female estuary perch (*Percalates colonorum*) caught in the Arthur River, Tasmania.

3.8 Spawning seasonality and development

In the Arthur River, GSI readings for female and male estuary perch peaked in December and dropped to resting levels during March and August (Table 3).

Table 3. Mean (\pm SE) GSI percentages for female and male estuary perch (*Percalates colonorum*) in the Arthur River.

Females			
Month	GSI %	SE	N
December (2014)	9.06	0.27	24
March (2015)	2.49	0.23	29
August (2015)	2.13	0.27	3

Males			
Month	GSI %	SE	N
December (2014)	6.47	0.50	7
March (2015)	1.93	0.40	8
August (2015)	0.7	0.01	10

GSI values were elevated in mature female and male estuary perch thus, verifying macroscopic staging techniques (Figure 17). For females, the highest GSI values ranged from 4 – 11% and were obtained in stage 3 ovaries. In contrast, stage 4 males produced the highest GSI values and varied from 2.9 – 8%. Modelled logistic curves could not be produced due to low captures of immature fish. Therefore, size or age at maturity could not be predicted with accuracy. Nevertheless, length at maturity appeared to vary between sex for estuary perch, with females maturing at larger sizes. Within the Arthur River, the smallest mature female was 29 cm FL with a GSI of 8% while the smallest mature male was 20cm FL with a GSI of 2.5% respectively (Figure 17).

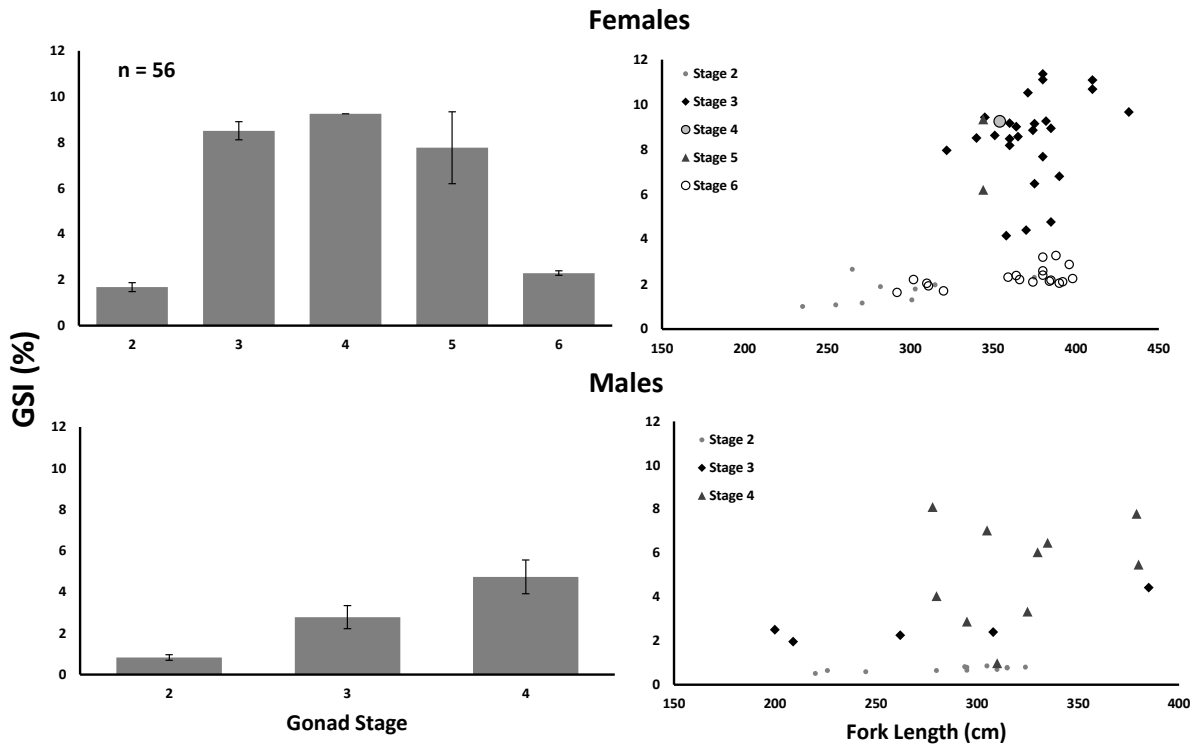


Figure 17. Gonadosomatic Index (GSI) for female and male estuary perch (*Percalates colonorum*) in relation to gonad stage and fork length (cm).

3.9 Oocyte size distributions

Estuary perch ovaries displayed tri-modal oocyte development in all maturity stages (Figure 18). In stage 3 (mature) ovaries, there were two modes of previtellogenic oocytes; one ranging between 0.2 and 0.4 mm in diameter and another ranging between 0.5 and 0.65 mm in diameter. There was also a larger mode of vitellogenic oocytes ranging between 0.7 and 0.9 mm in diameter (Figure 18). In stage 4 (ripe) ovaries, the group of vitellogenic oocytes became larger with some oocytes continuing to develop into hydrated (~1 mm diameter) oocytes. In stage 5 (ovulating) ovaries, more vitellogenic oocytes developed into larger, hydrated ova which were beginning to be ovulated. There was also additional size developments in previtellogenic oocytes with 0.5 – 6.5 mm diameter oocytes becoming more frequent. (Figure 18).

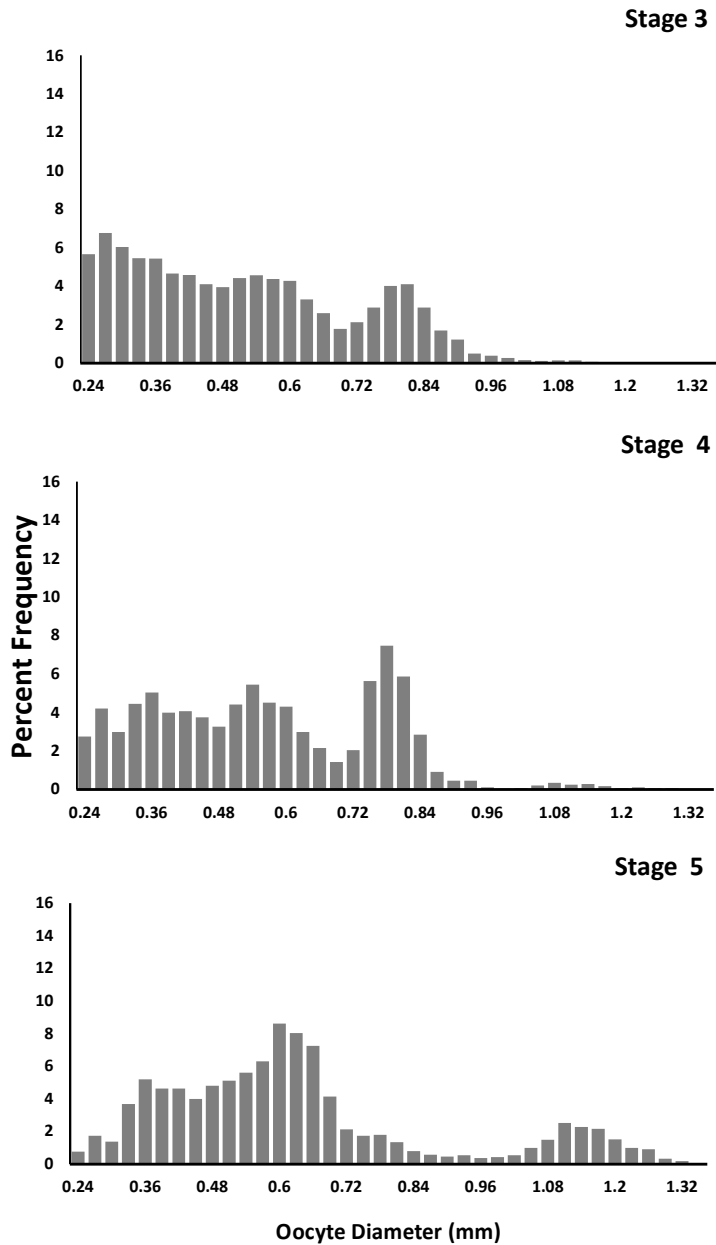


Figure 18. Oocyte size frequency distributions for stage 3 (mature), stage 4 (ripe) and stage 5 (ovulating) estuary perch (*Percalates colonorum*) females caught in the Arthur River, Tasmania.

3.10 Fecundity

3.10.1 Ovarian fecundity

Total ovarian fecundity for estuary perch ovaries in the Arthur River varied from 897, 367 to 2, 458, 425 (mean = 1, 547, 737 \pm 134, 730) (Figure 19). There was a significant linear relationship between ovarian fecundity and FL ($r = 0.71$, $n = 19$, $P = 0.001$). The relationship between ovarian fecundity and FL was best described linearly ($R^2 = 0.50$) rather than power regression ($R^2 = 0.48$).

3.10.2 Batch fecundity

Batch fecundity for estuary perch ovaries in the Arthur River ranged from 100, 226 to 228, 199 (mean = 164, 408 \pm 7, 839) (Figure 19). There was a significant linear relationship between batch fecundity and FL ($r = 0.76$, $n = 19$, $P = <0.001$). Similarly to ovarian fecundity, the relationship between batch fecundity and FL was best described linearly ($R^2 = 0.58$).

3.10.2 Number of potential batches

The number of potential batches varied from 6 (37cm FL) to 14 (38cm FL) (mean = 9.1 \pm 0.7) for estuary perch in the Arthur River. There no significant correlation between the number of potential batches produced and FL ($P = 0.27$).

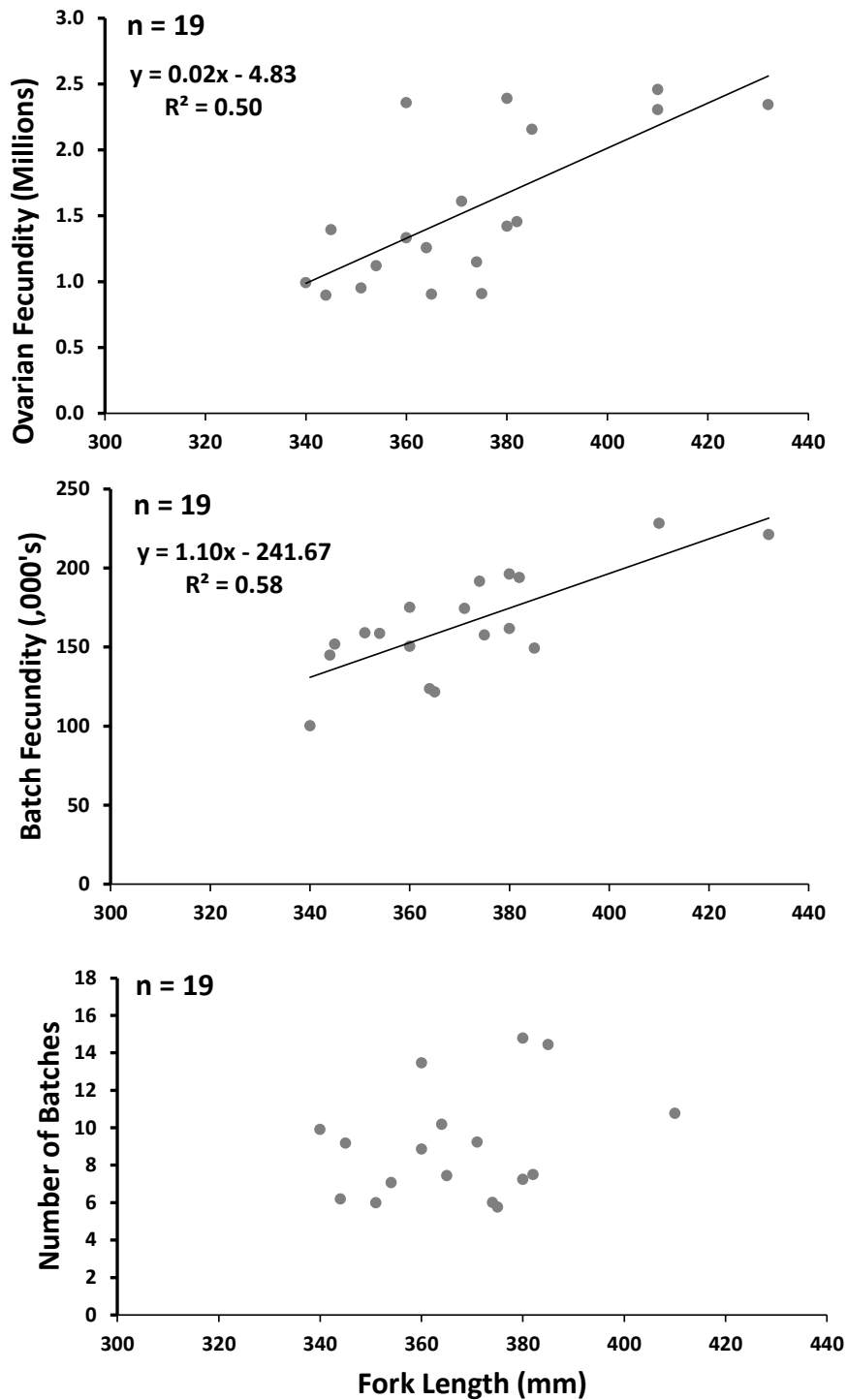


Figure 19. Relationship between ovarian fecundity, batch fecundity and the number of potential batches in relation to fork length (mm) for stage 3 female estuary perch (*Perca latipes*) caught in the Arthur River, Tasmania.

3.11 *Arthur River spawning time and parameters*

As peak spawning was identified to occur during December, salinity and temperature was profiled during this period. The lower, mid and upper estuarine areas of the Arthur River displayed vertical stratification in salinity and temperature during the 2014 spawning period (Figures 20 and 21). The depth of the halocline in the lower and mid estuary was around 2m where there was a sharp increase in salinity from slightly brackish (<3 ppt) to saline waters (30 ppt). In the upper estuary the halocline was deeper at around 4m where there was a sharp increase in salinity from fresh (<1 ppt) to relatively saline waters (25 ppt). The depth of the thermocline was deeper compared to the halocline at ~4m in the lower to mid estuary and 6m in the upper estuary. Temperature decreased with depth from 18 to 14°C throughout the estuary. Spawning estuary perch were caught in salinities ranging from 25 – 32 ppt and in temperatures of ~15 – 18°C.

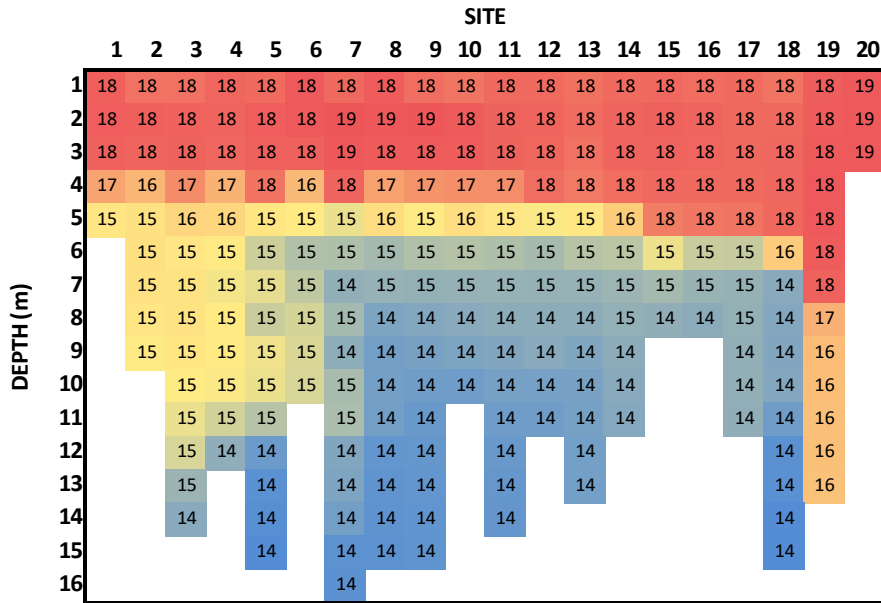


Figure 20. A water column profile showing changes in temperature (°C) in relation to depth for all sampling sites (Figure 1) in the Arthur River, December 2014. Figures relate to maximum recorded temperature (°C) at 1 m depth intervals (Red = high, Yellow = intermediate and blue = lowest temperatures recorded).

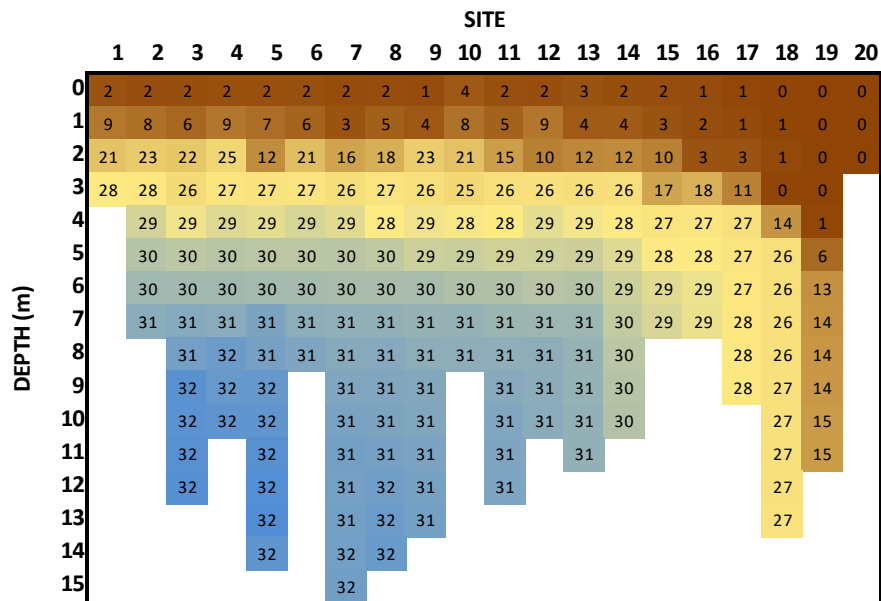


Figure 21. A water column profile showing changes in salinity in relation to depth for all sampling sites (Figure 1) in the Arthur River, December 2014. Figures relate to maximum recorded salinity (ppt) at 1 m depth intervals (Brown = freshwater, Yellow = brackish and blue = saline).

4.0 Discussion

4.1 *Spawning capacity of the Arthur River*

This study is the first to confirm that the estuary perch population in the Arthur River is reproductively active and capable of self-recruitment. Reproductive activity was highest in December and is likely to represent peak spawning activities for the species in the Arthur River as GSI values obtained during this period were similar to those reported for estuary perch in Victoria (Walsh et al. 2011). Estuary perch typically spawn in winter to early spring in mainland rivers (Walsh et al. 2011) thus, the summer spawning period highlighted in this study is noticeably delayed. However, it is possible that spawning activity may have occurred earlier or continued into later months as monthly sampling was outside the scope of this study. As such, the spawning duration in the Arthur River stock remains unclear.

The delayed spawning is probably explained by latitudinal climate differences in temperature and rainfall. Environmental spawning cues for teleosts include salinity, temperature, photoperiod and food availability (Quintana et al. 2004, Bhattacharyya and Maitra 2006, Rideout et al. 2005). In winter, the Arthur River estuary typically displays temperatures as low as 5 -10°C and is known to become homogeneously fresh during high rainfall events (Beard et al. 2008). Unlike NSW and Victorian stocks, temperatures and salinities observed in the Arthur River during winter and spring are not suitable for estuary perch spawning (Trnski et al. 2005), providing the most likely explanation for the spawning delay.

Walsh et al. (2011) found that a major spawning cue for estuary perch lays within the spawning habitat, specifically structurally complex wooden debris adjacent to deep saline

drop-offs. These structures provide protection from predators; deliver abundant amounts of food and enable eggs to disperse with deep water tidal flows (Walsh et al. 2011). While estuary perch optimal spawning parameters are not well documented, they are believed to be similar to Australian bass (Trnski et al. 2005). Australian bass larvae prefer salinities of 25 – 35ppt and temperatures of 18°C (Trnski et al. 2005). Estuary perch have previously been reported to spawn in salinities between 10 - 35ppt (McCarragher and McKenzie 1986, Walsh et al. 2011). In this study, ovulating females and milted males were caught in temperatures ranging from 15 - 18°C and salinities between 20 – 35‰. These temperatures and salinities were deemed adequate for successful estuary perch spawning in the Arthur River and were found as far as the salt wedge extended, 14km into the upper estuary. With an abundance of complex wooden debris adjacent to deep saline waters and adequate temperatures, the Arthur River appears to optimise the spawning potential of estuary perch. However, it is noted that the annual anoxia of the River during summer (Beard et al. 2008) may seriously reduce spawning potential.

This study highlighted tri-modal patterns of oocyte development in estuary perch ovaries, suggesting that the species is a group synchronous spawner. These results are compatible with previous research by Walsh et al. (2011). Mean batch fecundity for estuary perch in the Arthur River was 164, 408 oocytes and ranged from 100, 226 (34 cm FL) to 228, 199 (41 cm FL) respectively. Considering the larger sizes sampled in this study, these estimates were substantially lower than previous batch fecundity counts by Walsh et al. (2011) and McCarragher and McKenzie (1986). The reasons for the observed low batch fecundities could not be determined however, Gray et al. (2000) note that relative fecundity can vary between populations of percichthyids (Gray et al. 2000). Despite this observation, it is

unlikely that spatial variation alone is responsible for the extent of the observed differences in fecundity. Batch fecundity is sensitive to numerous environmental factors and is known to vary inter-annually in many species (Zamarro 1991, Duponchelle et al. 2000, Murua et al. 2006). Traditional batch fecundity estimates are usually based on hydrated oocyte counts (Zwolinski et al. 2001). The final hydration stage in oocyte development is rapid (hours), thus batch fecundity could vary considerably among individuals at different points during the spawning season (Fitzhugh et al. 2008). Assessing reproductive potential can be a challenging task and although total ovarian fecundity is often overlooked as a tool for reproductive output, it can be used to determine oocyte reserves (Loke-Smith et al. 2012).

Walsh et al. (2011) illustrated that in order to fully assess the reproductive potential of estuary perch, the number of times individuals may spawn during the reproductive season must be determined. Two methods are commonly used to determine spawning frequency: the “hydrated oocyte” method (DeMartini and Fountain 1981) where the proportion of ready-to-spawn (hydrated) fish is used to calculate the spawning frequency of the stock, and the “postovulatory follicles” (POF) method (Hunter and Goldberg 1980) where POFs are aged to determine the frequency of spawning events. The former requires persistent sampling and assumes stock synchronism during spawning, while the latter requires known ages of POF. Since both methods are more suited to r-selected species with stable spawning patterns (Stratoudakis et al. 2006), neither were applied in this study.

Based on the assumptions that all vitellogenic oocytes are released in the spawning season and pre-vitellogenic oocytes are not continually recruited, matured and spawned, between 6-14 spawning events are possible for one individual. However, more research is needed to validate these assumptions. The number of potential batches did not correlate with FL,

suggesting that while batch size increases in larger fish, the numbers of potential batches remain constant. Estuary perch are erratic spawners and unfavourable environmental conditions are known to delay or even prevent spawning (McCarragher and McKenzie 1986, Walsh et al. 2011). As oocytes in advanced stages have been found in the spent ovaries of estuary perch (Walsh et al. 2011), unpredictable proportions of unspawned oocytes could potentially be re-absorbed after a spawning period and the number of batches may vary inter-annually along with batch fecundity. Nevertheless, these estimates provide the first insight into the spawning potential of estuary perch in the Arthur River.

4.2 Population estimate

Mark-recapture techniques have evolved since the original single-census Peterson's Mark-recapture method (Petersen 1896). Many models have since been developed with new assumptions, each better suited to a different scenario (Cowley and Whitfield, 2001, de Azevedo Carvalho et al. 2013). While choosing the appropriate model can be difficult, Cowley and Whitfield (2001) suggests that the best suited model is usually the simplest one where all assumptions can be met. Therefore, for the purpose of this study, the original Peterson index was used to provide first hand estimates of estuary perch population size in the Arthur River. The basic assumptions highlighted by Cowley and Whitfield (2001) relevant to the Arthur River include:

1). *During the study no new individuals were born or immigrated into the population.*

Since the Arthur River population is expected to be the last in Tasmania, immigration of new recruits is not possible. Mark-recapture experiments were conducted after the 2014-2015 spawning period for estuary perch and therefore no additional recruitment could have

occurred. In future monitoring programs this assumption may not be met and additional tags will be needed.

2). No individuals died or emigrated from the population.

Estuary perch rarely move between rivers and will only migrate short distances with extreme flood events (Walsh et al. 2011). Since there are no neighbouring rivers which support estuary perch it is assumed that no emigration occurs in the Arthur River. Natural mortality in estuary perch populations has been estimated at approximately 0.36 y^{-1} (Taylor 2010). Estuary perch are currently protected from fishing in Tasmania and fishing mortality is limited to the select individuals taken for the purpose of this study. Neither the numbers euthanised in this study nor natural mortality is expected to violate the assumption of mark-recapture or affect population estimates.

3). Tagged individuals randomly mix with untagged individuals and the chances of being caught are equal between tagged and untagged fish.

Individuals were tagged during spawning aggregations and left to randomly mix for 2 months before sampling. Tagged fish appeared to mix freely with schooling fish on survey trips.

4). Individuals do not lose their tags and are easily distinguished from non-tagged fish.

Fish were double tagged with T bar tags to decrease the likelihood of tag loss. No recaptured individuals displayed signs of tag loss or infection from tagging processes.

5). Recapture rates of tagged and untagged individuals are high enough to support an accurate estimate.

With a recapture rate of <5% and only two sampling trips it is likely that this assumption was violated during this study.

Peterson mark-recapture population assessments are prone to underestimation when high recapture rates occur in conjunction with low sampling frequency (Pine et al. 2003).

Continuous sampling was outside the scope of this project and as such the population estimate of 1, 594 could underestimate the true population. The current estimate does not include fish smaller than 22cm FL (gear selection) and therefore is most likely conservative.

The large confidence intervals surrounding the population estimates suggest that these assessments must be viewed with caution. Nevertheless, this population estimate is the first of its kind in Tasmania and should be used as a baseline for future monitoring.

4.3 *Episodic recruitment*

Sampled catches from the Arthur River were dominated (68%) by three strong age classes: 12, 13 and 14 years. Missing, weak and dominant age classes indicate high inter-annual recruitment variability in the Arthur River. Episodic recruitment has previously been observed in estuary perch populations in Victoria and NSW (Walsh et al. 2010), as well as the closely related Australian bass (Harris 1985). Recruitment variability is also apparent in other long-lived estuarine species such as black bream (*Acanthopagrus butcheri*) (Morison et al. 1998) and barramundi (*Lates calcarifer*) (Staunton-Smith et al. 2004) and is usually a consequence of the harsh abiotic nature of the estuaries (Halliday et al. 2008). The specific causes of episodic recruitment in estuarine fish can be difficult to determine, differ between species and are often found to be related to environmental rather than human factors (Feyrer et al. 2007). Seasonally anoxic estuaries which coincide with the spawning times of

teleosts characteristically causes inter-annual recruitment variability (Breitburg et al. 2003, Breitburg 1994). For example, low DO can increase egg mortality and reduce the survival of early life stages of black bream, leading to marked differences in annual recruitment (Nicholson et al. 2008). The Arthur River often reaches anoxic states in saline waters during spawning periods (Beard et al. 2008) and since estuary perch eggs are released into benthic saline areas (van der Meulen et al. 2014), low DO could be an important factor contributing to episodic recruitment. More research is needed to determine the causes of anoxia, how low DO can impact recruitment of estuary perch and whether the anoxic nature poses an ecological risk to estuary perch in the Arthur River.

Freshwater flows can either negatively or positively influence the recruitment of estuarine fish (Haddy and Pankhurst 2000, Staunton-Smith et al. 2004, Walker and Neira 2001,). Strong year classes for estuary perch have been related to high freshwater flows during the spawning season (Walsh et al. 2011). In this study, strong year classes did not coincide with high rainfall events (data not presented: www.bom.gov.au/climate/data: site no. 091223, 091099 and 091357) and the specific causes for the observed strong year classes could not be determined. The abiotic nature of the Arthur River is complex, and recruitment variability is probably controlled by multiple interrelated factors including DO, salinity and larval food availability. The long-term survival of estuary perch in the Arthur River will continue to depend on the reproductive activity of strong age classes until multiple consecutive successful spawning seasons replenish the stock to stable levels. Re-stocking is used successfully in Victoria (DEPI 2014, Torrens et al. 2002) to enhance estuary perch fisheries and could prove advantageous in the Arthur River by increasing the strength of juvenile age classes after spawning periods with extreme anoxia. The additional restocking

of at least one other waterway which supported estuary perch in the past is also recommended as this population may act as a reserve in the event of an ecological crash in the Arthur River. Long-term stocking benefits include improving the conservation status of estuary perch and trialling freshwater land-locked stockings similar to those in Victoria (DEPI 2014) to enhance inland recreational fishing in Tasmania. Due to the restricted distribution, slow growth and uncertain recruitment variability of estuary perch, it is recommended that the Tasmanian conservation status be listed as “threatened” and existing no-take regulations continue to be enforced.

4.4 Age and growth

Age is one of the most influential biological variables used in stock assessments and validation is essential in ageing studies (Campana 2001). The methods used for age validation are diverse, but the most widely used and cost-effective method is marginal increment analysis (MIA) (Campana 2001). MIA relies on monthly samples of otoliths over at least two yearly cycles (Campana 2001) and thus, age validation was not in the scope of this study. Age validation (including MIA) has been accomplished with Victorian and NSW estuary perch otoliths and increments were found to be deposited annually during spawning periods (Walsh et al. 2010). Since increment formation is influenced by environmental factors such as temperature, photoperiod and food availability, the timing of these formations may vary spatially between populations (Taylor and Weyl 2013, Wilson and McCormick 1997, Anderson et al. 1992). Due to latitudinal differences between stocks, it was assumed that increment formation would occur during the summer spawning period for estuary perch in the Arthur River.

Estuary perch displayed sex related differences in growth with females growing faster and reaching greater asymptotic lengths than males. Similar findings have previously been reported for estuary perch (Walsh et al. 2010) and Australian bass (Harris 1987). The Arthur River is situated at a greater latitude than NSW and Victoria resulting in lower temperatures. While Walsh et al. (2010) reported no difference in the growth rates of estuary perch between rivers in Victoria and NSW, the growth rates of estuary perch in the Arthur River appear to be considerably slower than these populations. While this may partially be accredited to a lack of young fish in this data set, latitudinal growth differences have been observed in many Australian species including Australian bass (Harris 1987), black bream (Sarre and Potter 2000) and sand whiting (*Sillago ciliata*) (Stocks et al. 2011) and is directly affected by temperature. In addition to lower temperatures, the capacity for rapid growth is severely limited for estuary perch in Tasmania as the growing season coincides with spawning (Conover 1990). Although true size at maturity was not determined, the smallest mature male and female individuals found in the Arthur River were larger than those reported in Victorian rivers (Walsh et al. 2011). Slower growth rates may contribute to larger lengths at maturity compared to Victorian stocks (Walsh et al. 2011).

4.5 Non-destructive ageing

Scales

While otoliths are the preferred and most widely utilised structures used to age percichthyids (Lopez Cazorla and Sidorkewicz 2011, Walsh et al. 2010, Harris 1987), declining stocks require the development of non-destructive ageing techniques. Clear annual checks, low readability scores and a high correlation with otolith age suggests that scales can be used to age estuary perch accurately up to 8 years of age however, are not suitable for older

fish due to age underestimates. Using scales to age long-lived teleosts can often result in underestimations (Lopez Cazorla and Sidorkewicz 2011, Taylor et al. 2012) and while most estuary perch scales displayed clear annual checks, separating these increments became difficult towards the edge of the scale. Increment grouping has previously been described as a primary cause of age underestimation in scales (Beamish 1987). Age underestimations can lead to overestimations in growth rates and optimistic predictions of stock resilience (Campana 2001). This was evident in our VB growth models when using scales, particularly for males where K was overestimated by over 60%. Furthermore, valuable information on missing cohorts in the population age structure was lost, giving the impression that recruitment was more stable than observed with otolith ageing. Therefore, scales are not suitable for the analysis of population age structures for estuary perch. In contrast, scales have been used successfully to age many short lived, fast growing species (Khan and Khan 2009, Meulen et al. 2013) and are preferred for their effortless collection, easy preparation and non-destructive approach. The accuracy and clarity of scales for <8 year old estuary perch is similar to that described for short-lived teleosts (Khan et al. 2015, Dulčić et al. 2011). Klumb et al. (2001) showed that scales often present clearer increments than otoliths in early life stages during periods of fast growth and can produce more accurate back-calculations. This suggests there is potential to accurately age juvenile estuary perch non-destructively, however, due to male and female growth discrepancies, knowledge of sex is an essential requirement and can only be obtained non-destructively during spawning periods. For example, a 30 cm female could be six years old while a male could be up to 14 years old. Overall, using scales in the absence of otoliths is risky, and age underestimations could result in overestimations in growth and mortality (Campana 2001).

Spines

Anal fin spines had the highest IAPE and were inferior to other structures used to age estuary perch. Overestimations were evident for younger individuals (<6 years), suggesting false annuli may have been present in sectioned fin spines, however, the general trend showed an underestimation of ages. Difficulties in the interpretation of fin spine annuli are not uncommon and can frequently result in underestimations (Brusher and Schull 2009, Khan et al. 2015, Maceina and Sammons 2006, Sipe and Chittenden 2001). As fish grow to larger sizes, the spine nucleus can be absorbed (vascularisation) and be replaced by a hole, often eliminating early growth bands in the process (Buckmeier et al. 2002). True age can be estimated in short lived species by calculating the diameters of early annuli in young fish prior to vascularisation and localising their positions in older fish (Zaboukas and Megalofonou 2007). However, this may be difficult to achieve in long-lived species due to slow growth abnormalities in the microstructure of fin spines (Kocovsky and Carline 2000). Merged annuli, crowding of distal banding and partially formed increments were evident in the spine structure of estuary perch and were the probable cause of age underestimation. Most of the problems associated with spine age estimates are not relevant to otoliths and despite the destructive method, they present robust age estimates for estuary perch.

4.6 *Back-calculations*

Both otolith and scale radii were positively correlated with fish length, supporting the major assumption of nearly all back-calculation models: *fish length is directly proportional to otolith and scale length* (Campana 1990).

Lee's phenomenon was evident as direct proportional back-calculations from female and male estuary perch otoliths and scales produced underestimated historical length-at-age data compared to raw data (Baumann et al. 2013). As a result, growth trajectories from back-calculated lengths presented higher growth rates (K) and higher asymptotic lengths (L_{∞}). Similar findings have been reported for comber (*Serranus cabrilla*) (Tserpes and Tsimenides 2001) and white-spotted char (*Salvelinus leucomaenis*) (Morita and Matsuishi 2001).

Scale back-calculations produced higher growth rates than otoliths for females ($K = 0.15$ vs. 0.13) and males ($K = 0.17$ vs. 0.13). Furthermore, growth rates from back-calculations did not differ between sexes ($K = 0.13$), while with scales, females displayed slower growth rates than males ($K = 0.15$ vs. 0.17). This contradicted what has been previously described for this species (Walsh et al. 2010), suggesting that back-calculations for estuary perch are unreliable. While older scales with deviated ages were removed to make annual increments in otoliths and scales more comparable, back-calculated lengths differed considerably between otoliths and scales. This implies that either the proportionality between increment widths differed among the two hard parts or interpretational errors occurred from the circuli of scales or both. Systematic differences among back-calculated lengths from scales and otoliths have previously been related to proportional variances between the first increments of scales and otoliths (Baumann et al. 2013). While corrections can be made to make scale length-at-age data more comparable with otoliths (Baumann et al. 2013), the unusual lack of variation between male and female growth rates and consistent underestimation of lengths-at-age suggest that back-calculations are inaccurate for estuary perch using the direct proportional method.

The otolith - fish length relationship which back-calculations depend upon can vary (Mosegaard et al. 1988, Reznick et al. 1989). For example, two fish of the same length may have significantly different otoliths sizes, depending on their growth rate (Francis 1990). In addition, somatic growth substantially slows in older fish while otolith size continues to increase without decelerating (Campana 1990). Therefore, the assumption that fish and otolith length are directly proportional may be violated in older individuals. These variations contribute to the uncertainty related to back-calculations, particularly in long-lived fish (Campana 2005).

While scale and otolith back-calculations produce unreliable length-at-age estimates for older estuary perch, there is still potential to apply back-calculations to younger fish. Many of the problems associated with back-calculations including Lee's phenomenon, variability between otolith and fish growth and fluctuating otolith sizes for similar length fish can be avoided by limiting data to young ages (Baumann et al. 2013), providing growth discrepancies are accounted for. Since scales can produce more distinguishable increments than otoliths in younger ages, they may reveal more accurate historical length estimates (Klumb et al. 2001). However, these lengths need to be sufficiently validated with raw length-at-age data for young estuary perch. Once validated, juvenile biochronologies can be developed to understand environmental effects on growth in early life stages. Also, length-at-age data can be produced for smaller sizes not selected by gear to expand early growth trajectories in current VB models.

5.0 Conclusion

As seen previously in NSW and Victorian populations (Walsh et al. 2010), an extreme case of episodic recruitment was confirmed in the Arthur River. Nevertheless, estuary perch are highly fecund, opportunistic spawners and have the added advantage of longevity, ensuring their resilience to seasons unfavourable for reproduction. This study has established a considerably delayed spawning season for estuary perch which was attributed to differences in latitudinal rainfall, water temperature and salinity. A summer spawning pattern shortens the growing season for estuary perch in Tasmania and along with latitudinal temperature differences, could result in slower growth compared to mainland populations. Scales and anal fin spines underestimate the age of estuary perch compared to otoliths. Consequently, ages can only be determined non-destructively for young individuals up to eight years of age using scales. Variances between somatic and otolith growth rates in older fish resulted in imprecise length-at-age back-calculations for estuary perch. Scale back-calculations also produced inaccurate length-at-age data, but this was more likely caused by crowding of distal annuli and reading difficulties. The problems associated with back-calculations in estuary perch may be resolved by limiting the data to younger ages (<8 years). The current population estimate of 1,594 is likely to be underestimated and further mark-recapture efforts are recommended to accurately quantify the population size in the Arthur River. Considering the slow growth and extreme recruitment variability of estuary perch in the Arthur River, as well as the restricted distribution in Tasmania, the results of this study suggest that current no-take policies should continue and the Tasmanian conservation status be listed as “threatened”.

6.0 References

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7.0 Appendices

Appendix A

Generating automated oocyte counts and size frequency distributions with image J

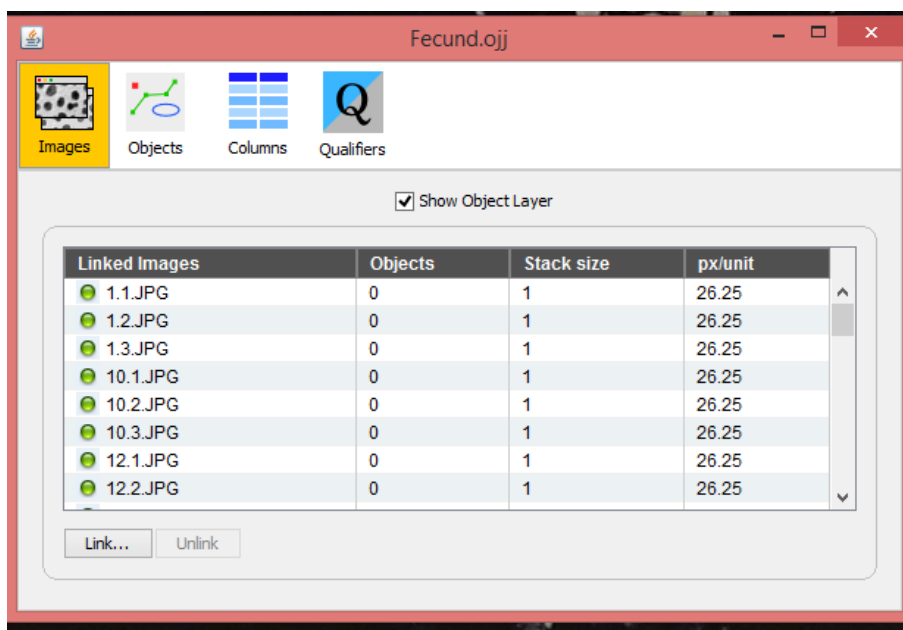
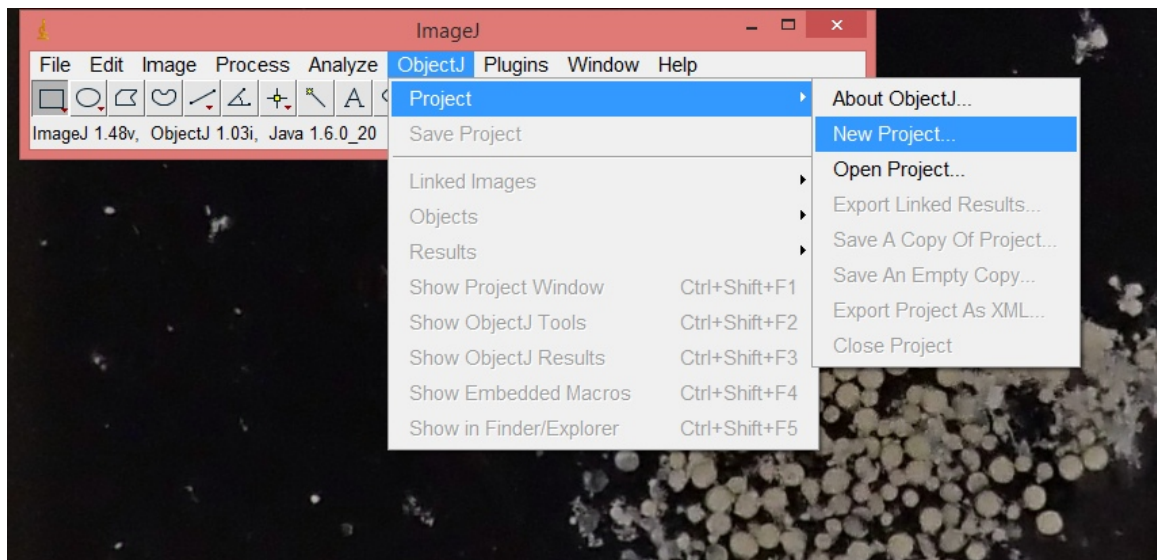
Step 1. Take photos of oocytes with a standardized magnification and take at least one photo with a scale bar. Make sure each photo has an ID corresponding with the fish ID in the image title.

Step 2. Download Image J (<http://rsb.info.gov/ij/index.html>) and Object J (<http://simon.bio.uva.nl/objectj/index.html>).

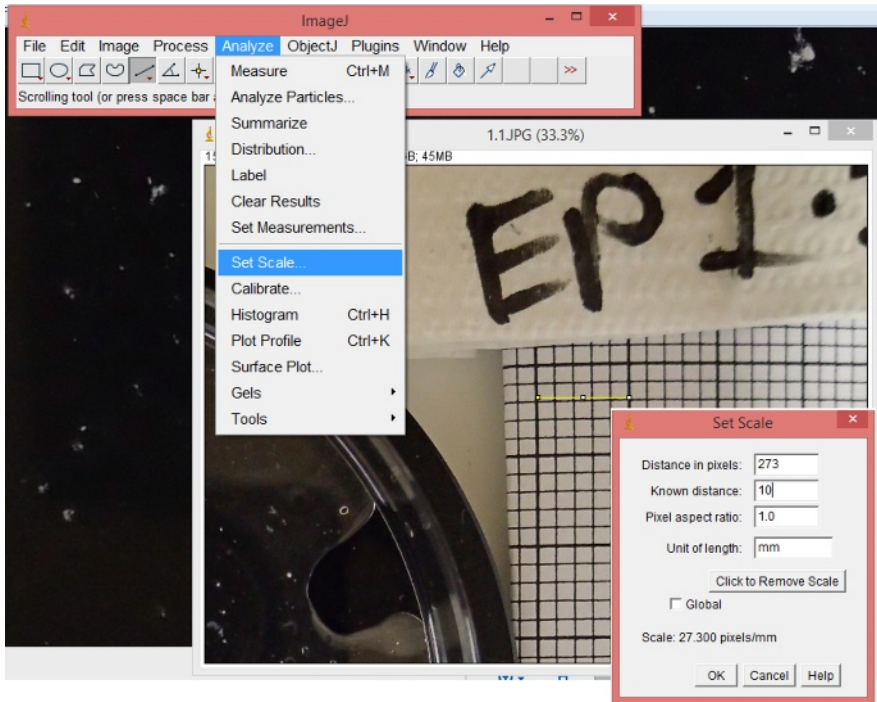
Step 3. Move the Object J file 'objectj_' into the image J plugins folder (program files > ImageJ > Plugins) to allow Object J to work in Image J.

Step 4. Store all images in one folder. Activate the Object J plugin by going "Plugins" > Object J on the Image J tool bar. Start a new project using the object j plugin (save as "project name.ojj"). This will bring up the Object J window. Click and drag all images to the

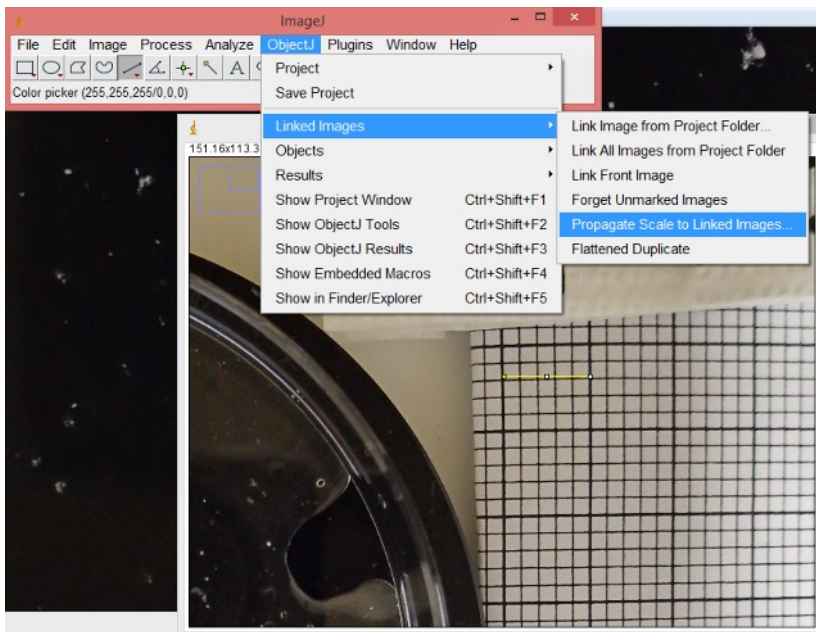
object J window. This will link all images in the project.



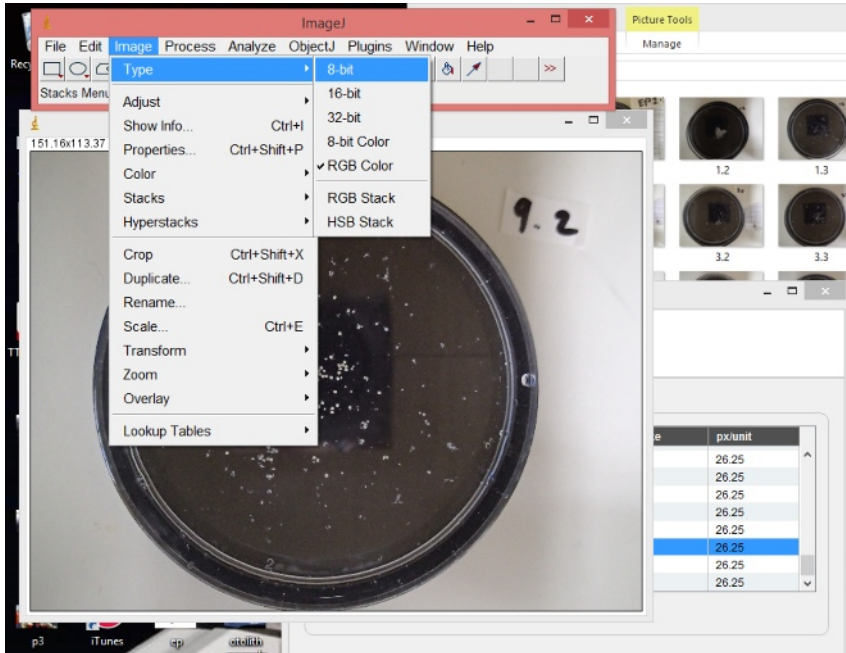
Step 5. Click on the image which includes a scale bar and set scale on image J by clicking on Analyse > Set Scale. Using the straight line selection tool, measure a known distance on the image and calibrate according to the pixel length by entering the known distance and the units in the “set scale” window.



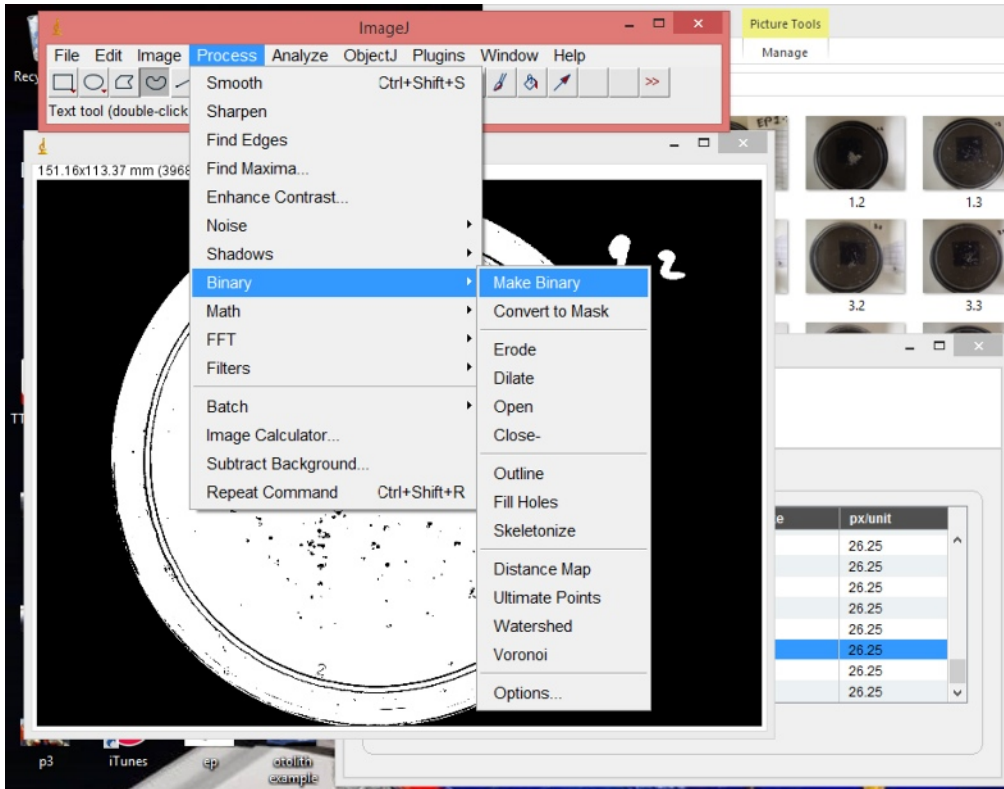
Step 6. Automatically apply this scale to all linked images by going Object J > Linked images > Propagate scale to linked images. This means you do not have to set the scale for each individual photo. Now you are ready to begin oocyte analysis.



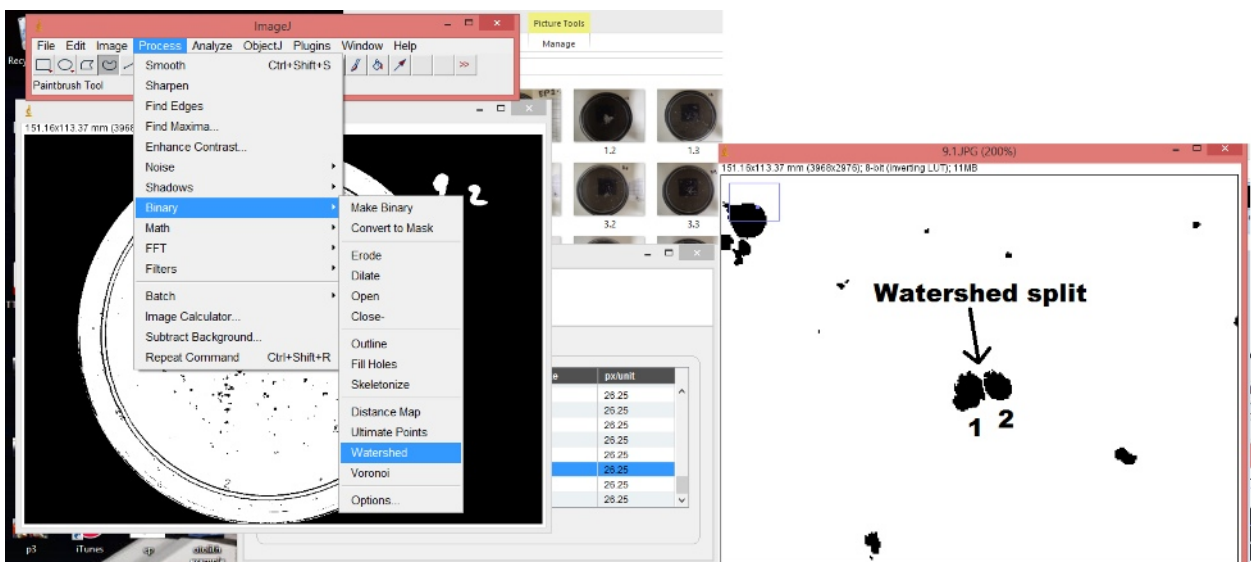
Step 7. Bring up your first image by double clicking it in the object J panel. Change the image type to 8-bit greyscale (Image > Type > 8-bit).



Step 8. Change to binary to bring dark images to foreground by going Process > Binary > Make Binary.

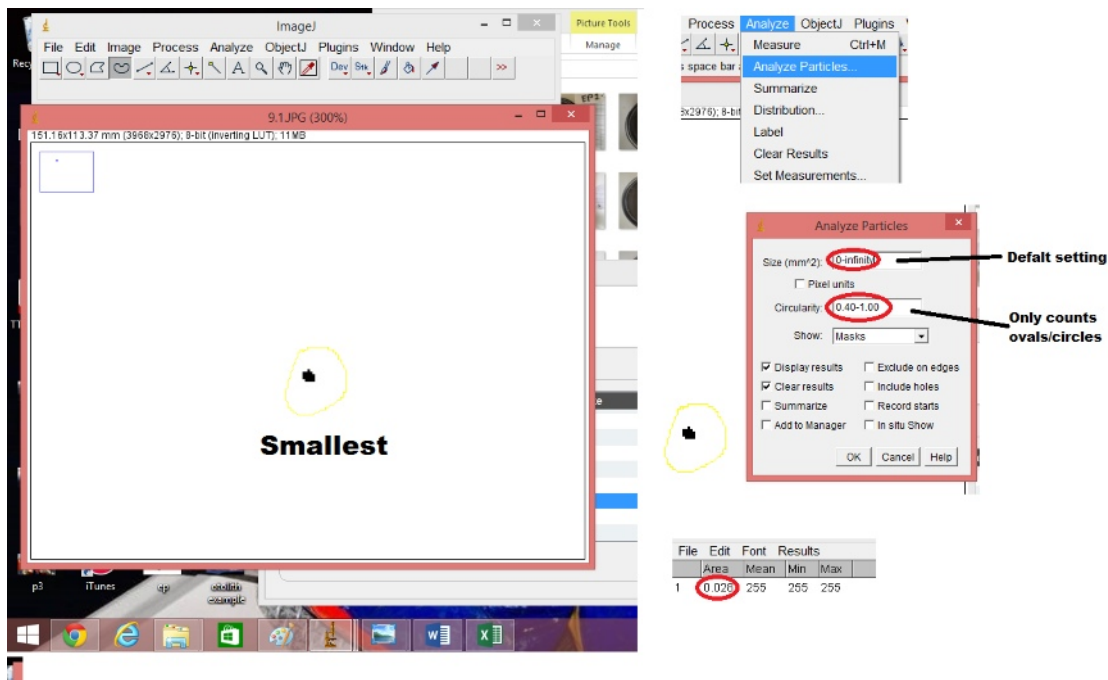


Step 9. Objects touching each other must be separated by selecting the watershed option under Process > Binary > Watershed.



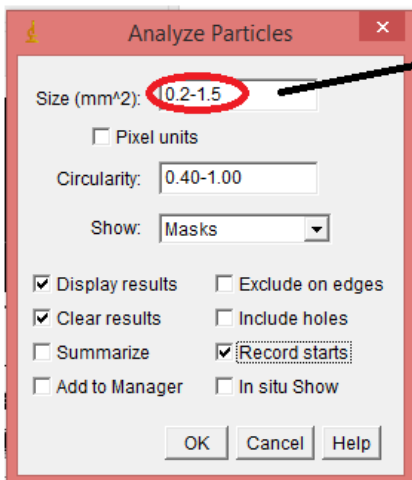
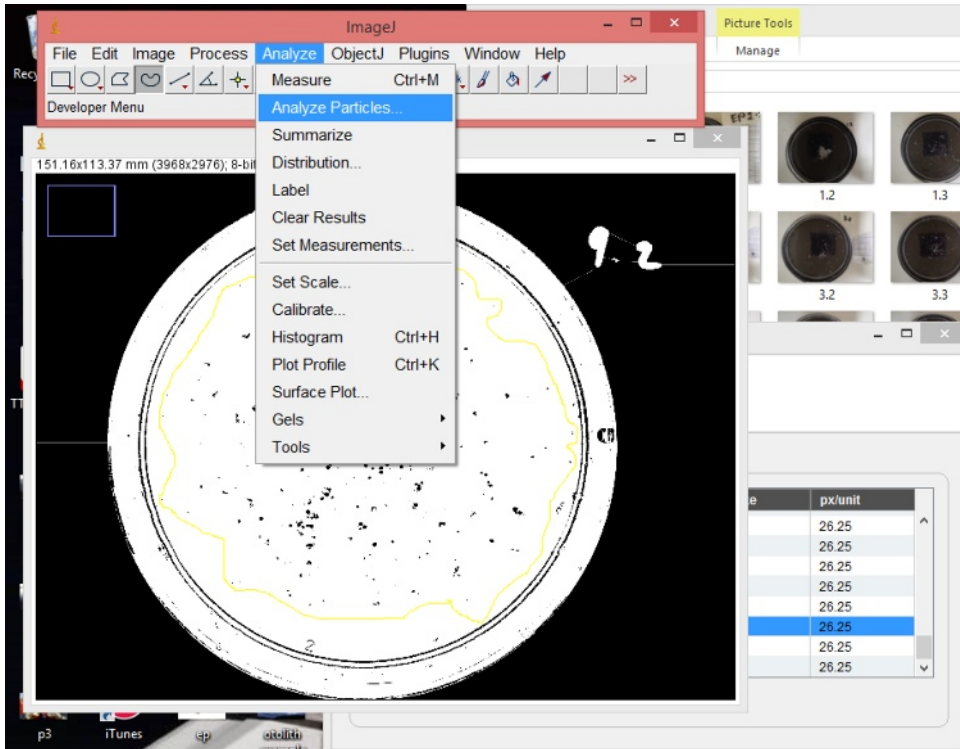
Step 10. Using the free-hand selection tool select the SMALLEST individual egg in the sample.

Step 11. Analyse this particle size by Analyse > Analyse Particles. Take note of the area of this particle so you can set a lower limit when automating particle counts (avoids counting miniscule sized non-oocyte particles in the image).



Step 12. Repeat step 9 and 10 for the largest sized particle. This is just to give yourself an indication of the size range you should be looking to count. You do not need to do this for every image and you may wish to vary the size range depending on the maturity stage of the fish.

Step 13. Now using the free-hand selection tool select all the particles you wish to count and measure.



Enter the lower and upper range (area) of oocytes. Only oocytes between this range will be counted and measured

Step 14. Analyze Particles. This will provide a spreadsheet of the number and area of each individual oocyte. To get the diameter you will have to transpose the equation:

$$\text{Area} = \pi * \text{radius}^2$$

Appendix B

Table 4. AoRSS Table of comparisons for female and male growth differences (VB growth modelling) between hard parts and constrained data. P values highlighted in red are considered significant ($\alpha = 0.05$).

AoRSS Comparisons			
Growth Comparisons (Females and Males) Using:	df	f	p
Otoliths	82	65.78	<0.001
Scales	76	13.79	<0.001
Back-Calculated Otoliths	978	475.62	<0.001
Back-Calculated Scales	637	49.63	<0.001
Back-Calculated Otoliths Constrained (Ages 5 -15)	1501	1.08	0.36
Back-Calculated Scales Constrained (Ages 5 -15)	444	1.15	0.33
Growth Comparisons Using:			
Otoliths and Scales (Females)	111	0.65	0.58
Otoliths and Scales (Males)	47	6.91	<0.001
Otoliths and Scales Constrained (Ages 5 - 15) Females	92	0.31	0.82
Otoliths and Scales Constrained (Ages 5 - 15) Males	44	5.73	<0.001
Back-Calculated Otoliths Vs Otoliths (Females)	693	6.07	<0.001
Back-Calculated Otoliths Vs Otoliths (Males)	367	5.73	<0.001
Back-Calculated Scales Vs Otoliths (Females)	547	2.95	0.03
Back-Calculated Scales Vs Otoliths (Males)	156	4.10	0.01
Back-Calculated Otoliths Vs Scales (Females)	1142	56.63	<0.001
Back-Calculated Otoliths Vs Scales (Males)	473	31.81	<0.001
Back-Calculated Otoliths Vs Otoliths Constrained (Ages 5 - 15) Females	446	5.02	<0.001
Back-Calculated Otoliths Vs Otoliths Constrained (Ages 5 - 15) Males	219	3.15	0.02
Back-Calculated Otoliths Vs Scales Constrained (Ages 5 -15) Females	812	10.82	<0.001
Back-Calculated Otoliths Vs Scales Constrained (Ages 5 -15) Males	314	24.95	<0.001

Table 5. Table of various von Bertalanffy growth models with respective parameters for Raw otoliths, scales and back-calculated otolith and scale lengths.

Table of models			
Raw Otoliths	L_{∞}	K	T_0
T_0 constrained all ages (Males)	35.43	0.16	0
T_0 constrained all ages (Females)	39.57	0.22	0
T_0 constrained ages 3 - 15 (Males)	32.76	0.21	0
T_0 constrained ages 3 - 15 (Females)	39.25	0.22	0
Unconstrained all ages (Males)	39.7	0.09	-4.1
Unconstrained all ages (Females)	45.62	0.11	-2.95
Unconstrained ages 3 - 15 (Males)	52.42	0.04	-7.2
Unconstrained ages 3 - 15 (Females)	41.9	0.15	-1.55
Raw Scales	L_{∞}	K	T_0
T_0 constrained all ages (Males)	37.66	0.18	0
T_0 constrained all ages (Females)	38.89	0.24	0
T_0 constrained ages 3 - 15 (Males)	37.07	0.18	0
T_0 constrained ages 3 - 15 (Females)	38.67	0.24	0
Unconstrained all ages (Males)	39.35	0.14	-1.1
Unconstrained all ages (Females)	42.4	0.14	-2.39
Unconstrained ages 3 - 15 (Males)	41.56	0.12	-1.72
Unconstrained ages 3 - 15 (Females)	40.82	0.16	-1.6
Back-Calculated Otoliths	L_{∞}	K	T_0
T_0 constrained all ages (Males)	29.2	0.24	0
T_0 constrained all ages (Females)	35.28	0.27	0
T_0 constrained ages 3 - 15 (Males)	29.44	0.26	0
T_0 constrained ages 3 - 15 (Females)	38	0.21	0
Unconstrained all ages (Males)	33.79	0.12	-2.52
Unconstrained all ages (Females)	43.28	0.12	-2.04
Unconstrained ages 3 - 15 (Males)	34.65	0.13	-2.12
Unconstrained ages 3 - 15 (Females)	42.35	0.13	-1.94
Back-Calculated Scales	L_{∞}	K	T_0
T_0 constrained all ages (Males)	32.04	0.26	0
T_0 constrained all ages (Females)	38.32	0.21	0
T_0 constrained ages 3 - 15 (Males)	32.04	0.26	0
T_0 constrained ages 3 - 15 (Females)	38.32	0.21	0
Unconstrained all ages (Males)	36.03	0.17	-0.88
Unconstrained all ages (Females)	42.05	0.15	-0.69
Unconstrained ages 3 - 15 (Males)	36.03	0.17	-0.88
Unconstrained ages 3 - 15 (Females)	42.72	0.15	-0.72