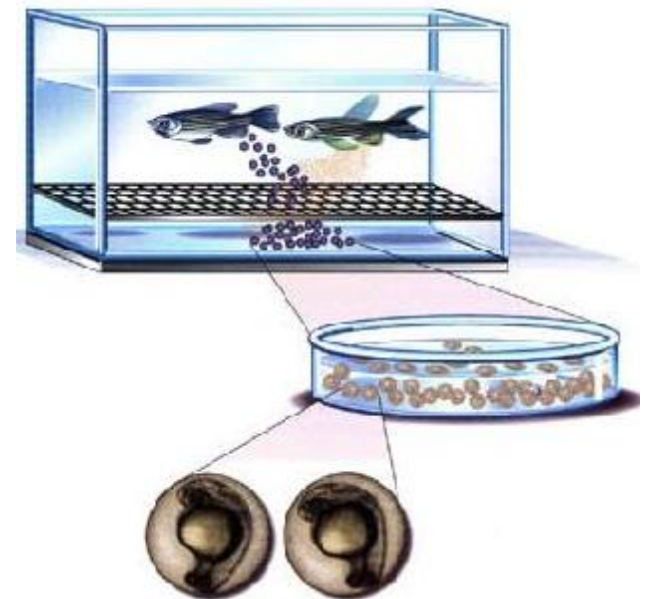
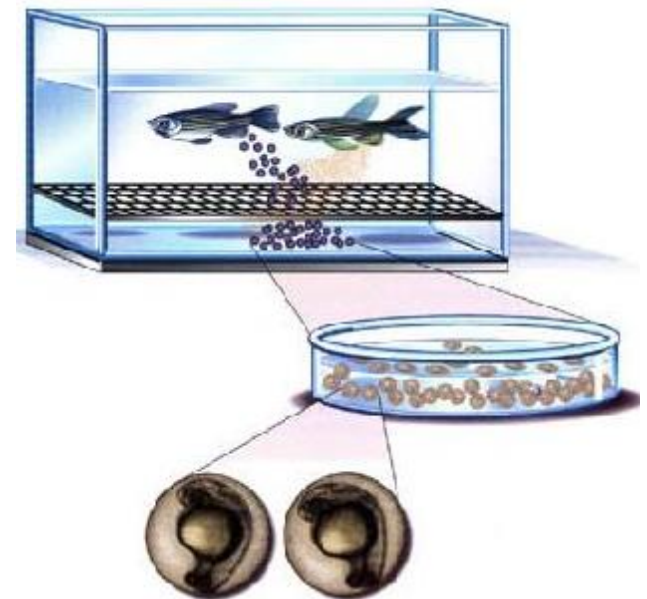


Zebrafish Spawning and Reproduction



Talk Outline

- Two stages to achieving reproductive success in the lab!
 - Sexing zebrafish
 - practical measures
 - morphological & behavioural characteristics
 - Species specific knowledge
 - making the right choices – improve reproductive output
 - management opportunities – easier for you!
 - inform on best welfare practices



SEXING ZEBRAFISH

Sexing Zebrafish

Whatever your experience you need to choose the best quality fish!



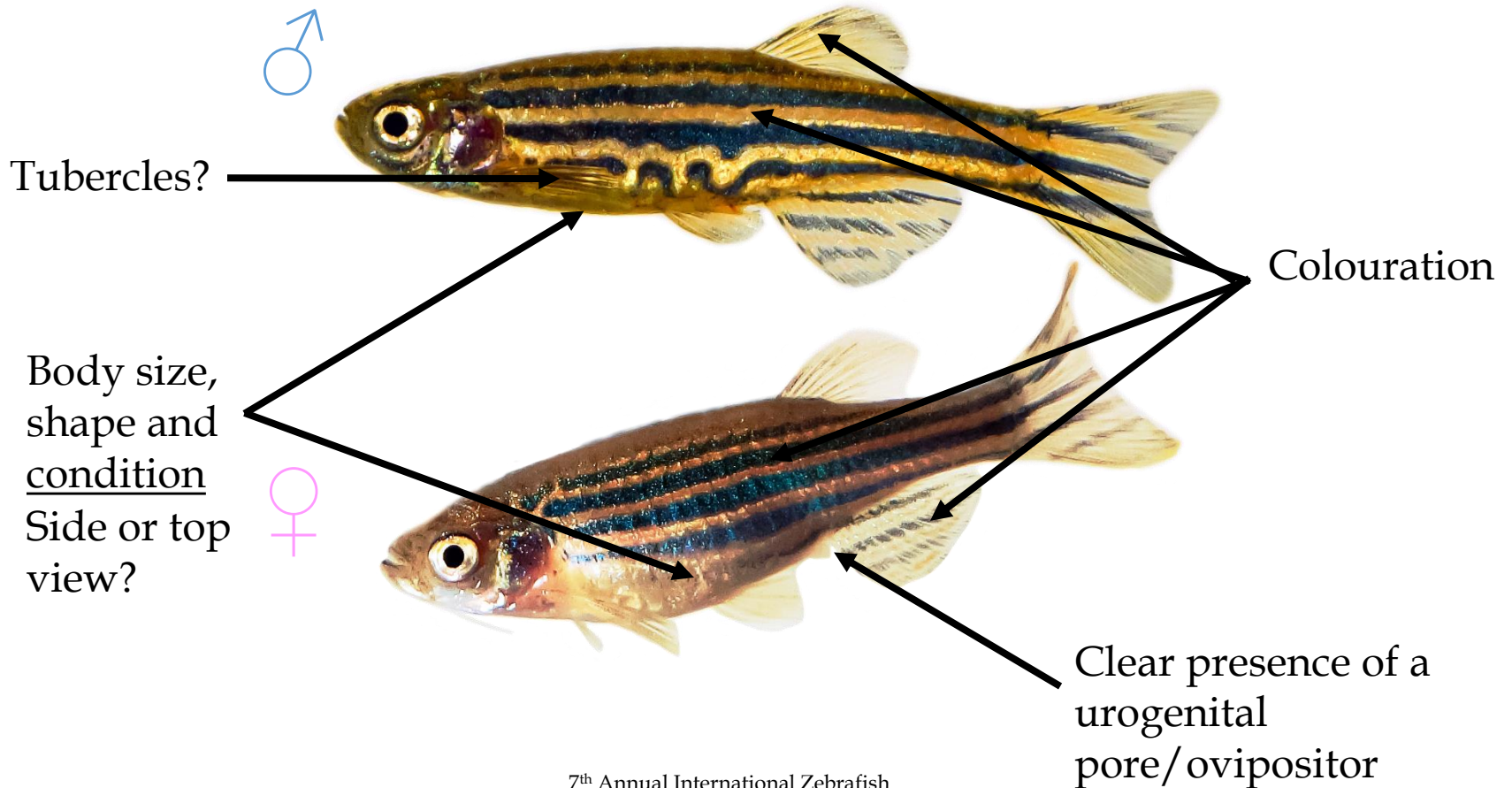
Picture courtesy of S. Mourabit
UoE 2017

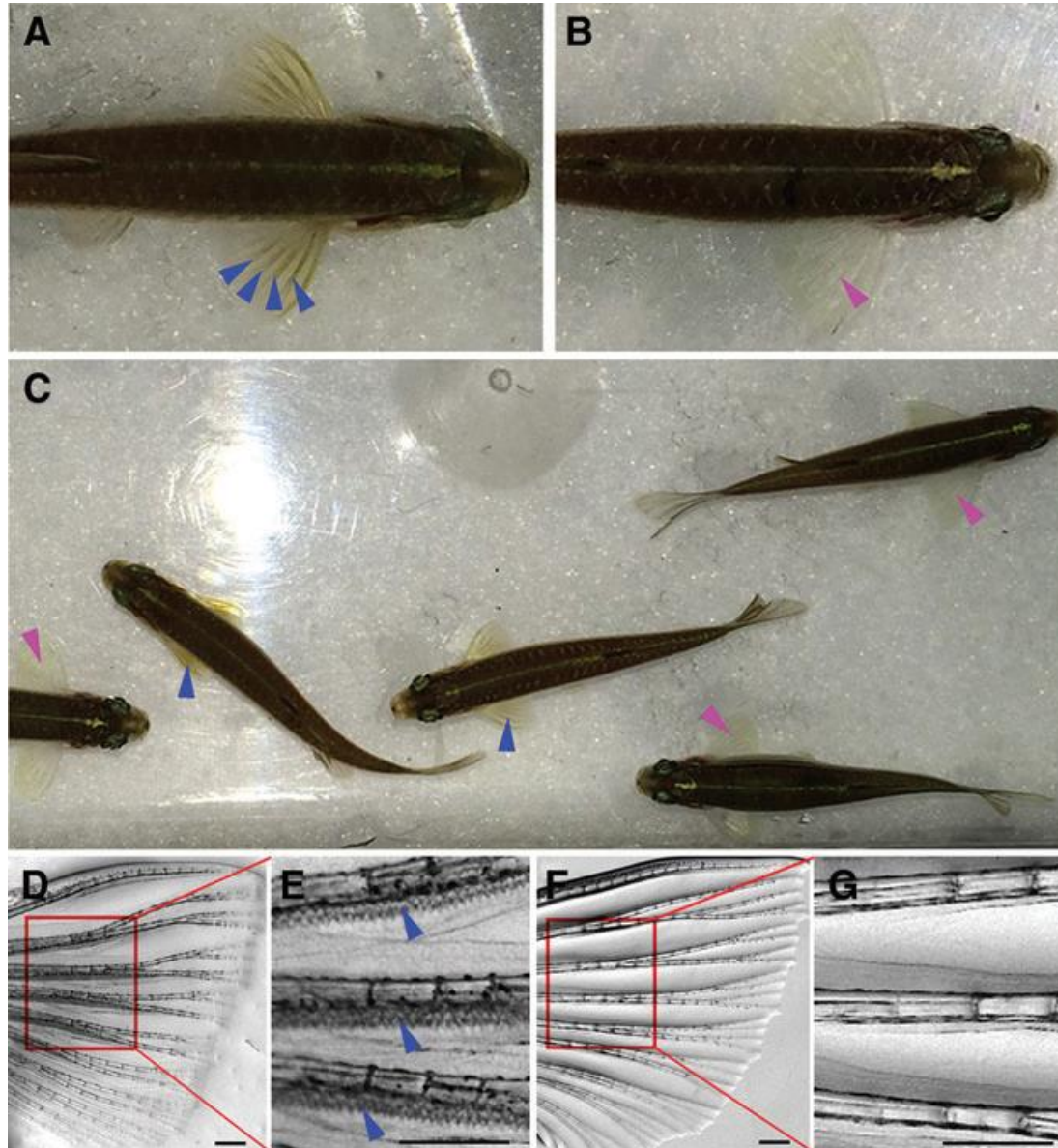
Sexing Zebrafish

- Practical measures before you start!
 - Make it easier for yourself!
 - give yourself time & space
 - set a cut off time in your facility?
 - have a large enough pool of fish to sex from
 - ensures quality!
 - don't try & sex fish from a densely populated tank of fish
 - smaller groups saves time & stress for the fish and you!
 - don't sex fish immediately after feeding
 - males can become almost as gorged as females
 - colouration differences between sexes most prevalent in the morning
 - Use your full tool-kit to distinguish between sexes
 - morphological & behavioural characteristics

Sexing Zebrafish

- Morphological characteristics to look for when selecting your fish
 - Sexual dimorphism weak in this species?

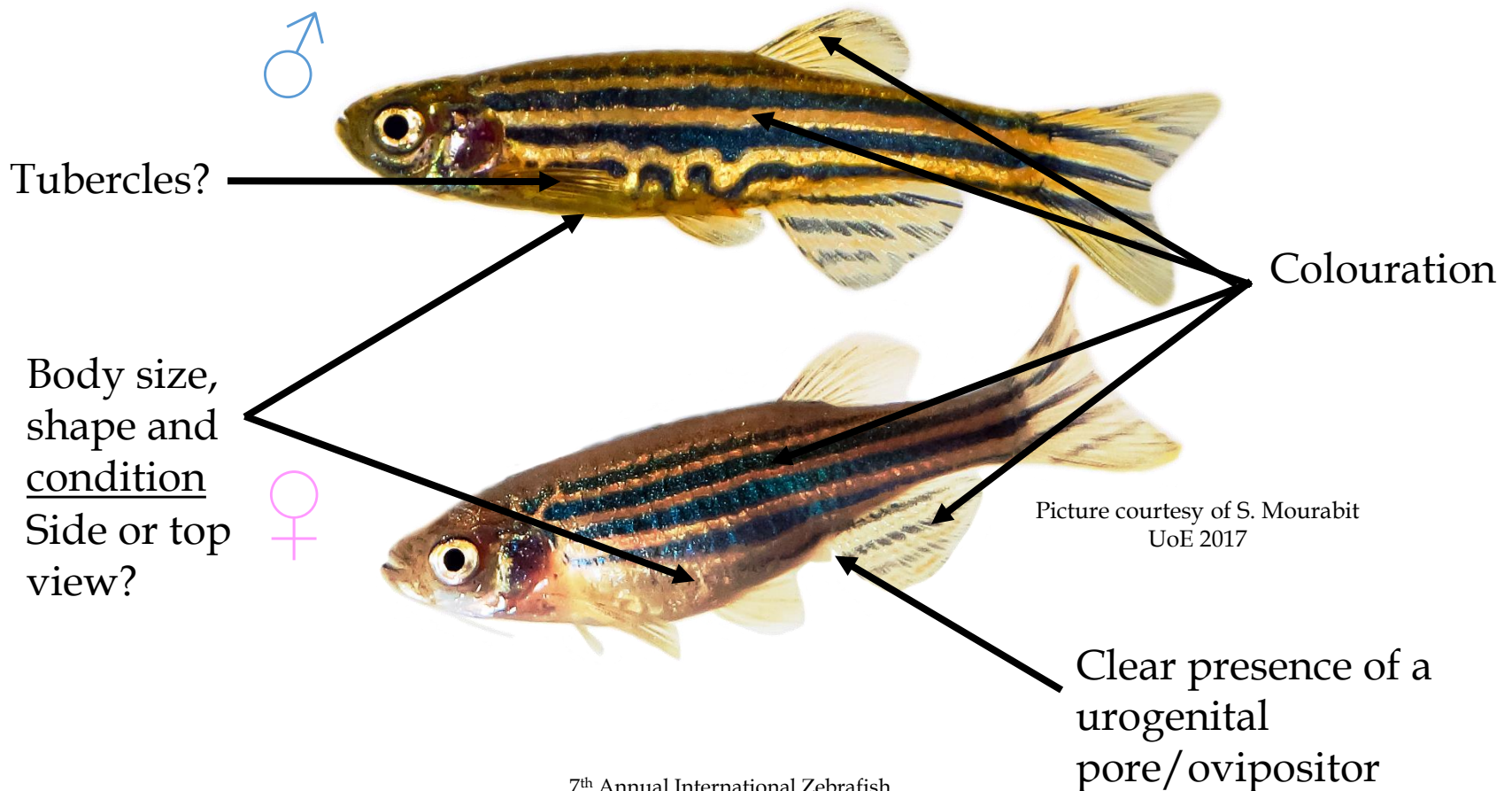




McMillan et al,
2014: Zebrafish

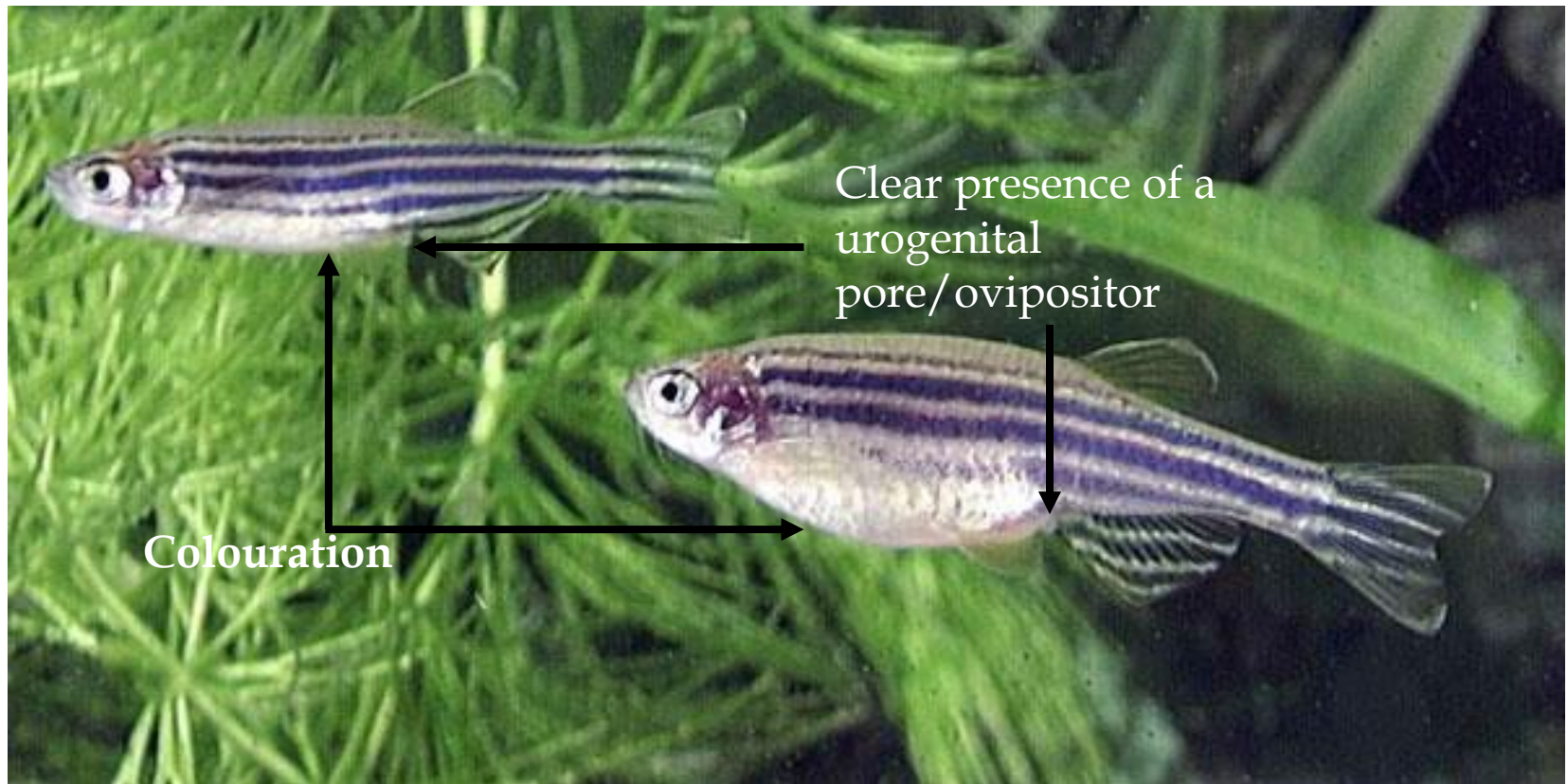
Sexing Zebrafish

- Morphological characteristics to look for when selecting your fish



Sexing Zebrafish

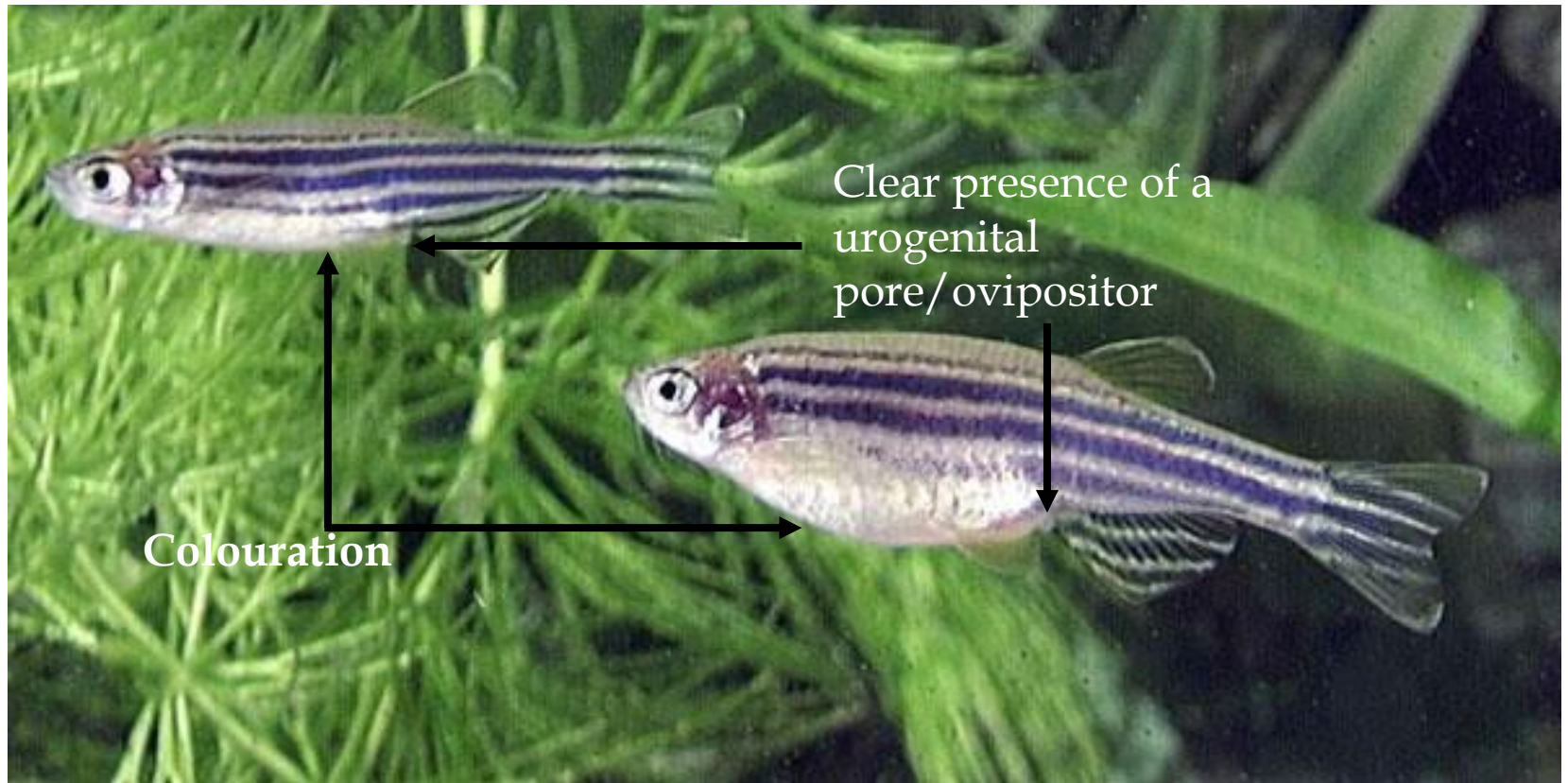
- Morphological characteristics to look for when selecting your fish



These fish are not a pair!

Sexing Zebrafish

- Morphological characteristics to look for when selecting your fish



These fish are not a pair!

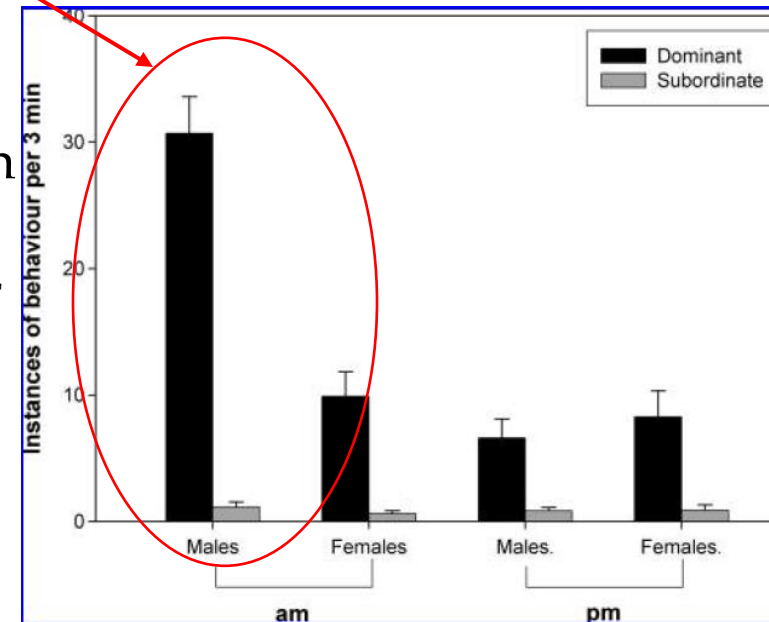
Don't always have perfect fish

- Behavioural characteristics to look for when sexing your fish

- Zebrafish show a lot of behavioural interaction

- notably aggression & territorial behaviour
- aggressive activity highest in the morning
- greater in males than in females

Paull et al., 2010. Dominance hierarchies in zebrafish (*Danio rerio*) and their relationship with reproductive success



- Spawning occurs at the onset of dawn

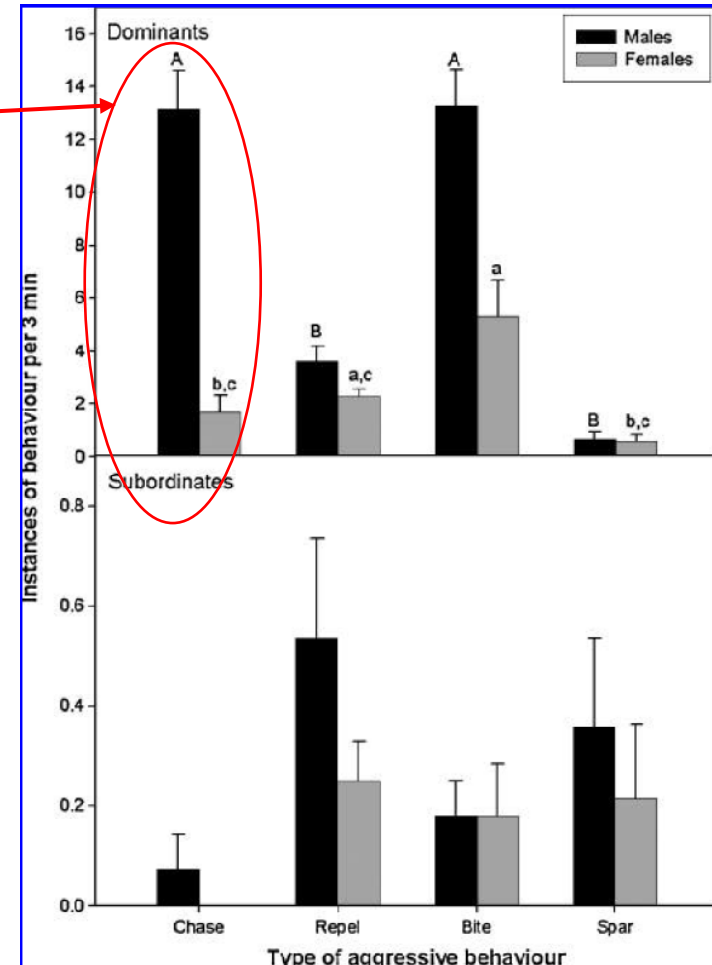
- in males this aggression is likely to function to facilitate spawning and/or control spawning activities between individuals

- Females can also be aggressive

- no temporal difference
- suggesting aggression may have a more general role in maintaining social structure in females
- some females are also observed to avoid physical interaction ...dependent on social setting!

- Behavioural characteristics to look for when sexing your fish

Behaviour	Description
Chase	Fish swims directly/aggressively towards another fish in the aquarium causing it to increase its speed (and possibly change direction) and actively pursues the fish.
Repel	When approached by another fish, the fish makes a sudden movement that causes the approaching fish to change direction, or the fish swims a short distance towards another fish causing it to suddenly change direction but then stops and immediately returns to its previous position (i.e. does not actively pursue it).
Bite	Fish clearly opens its mouth and bites other fish, often on the tail or ventral fins. This often occurs during, or immediately following, a chase and/or repel
Spar	Fish slowly approaches another fish with its fins flared and offers his/her body flank to the other fish gently oscillating its body with the effect of making it appear a larger size. The opposing fish either moves off or reciprocates the behaviour sometimes resulting in elaborate circling motion between the two fish which can last from a few seconds though to several minutes.

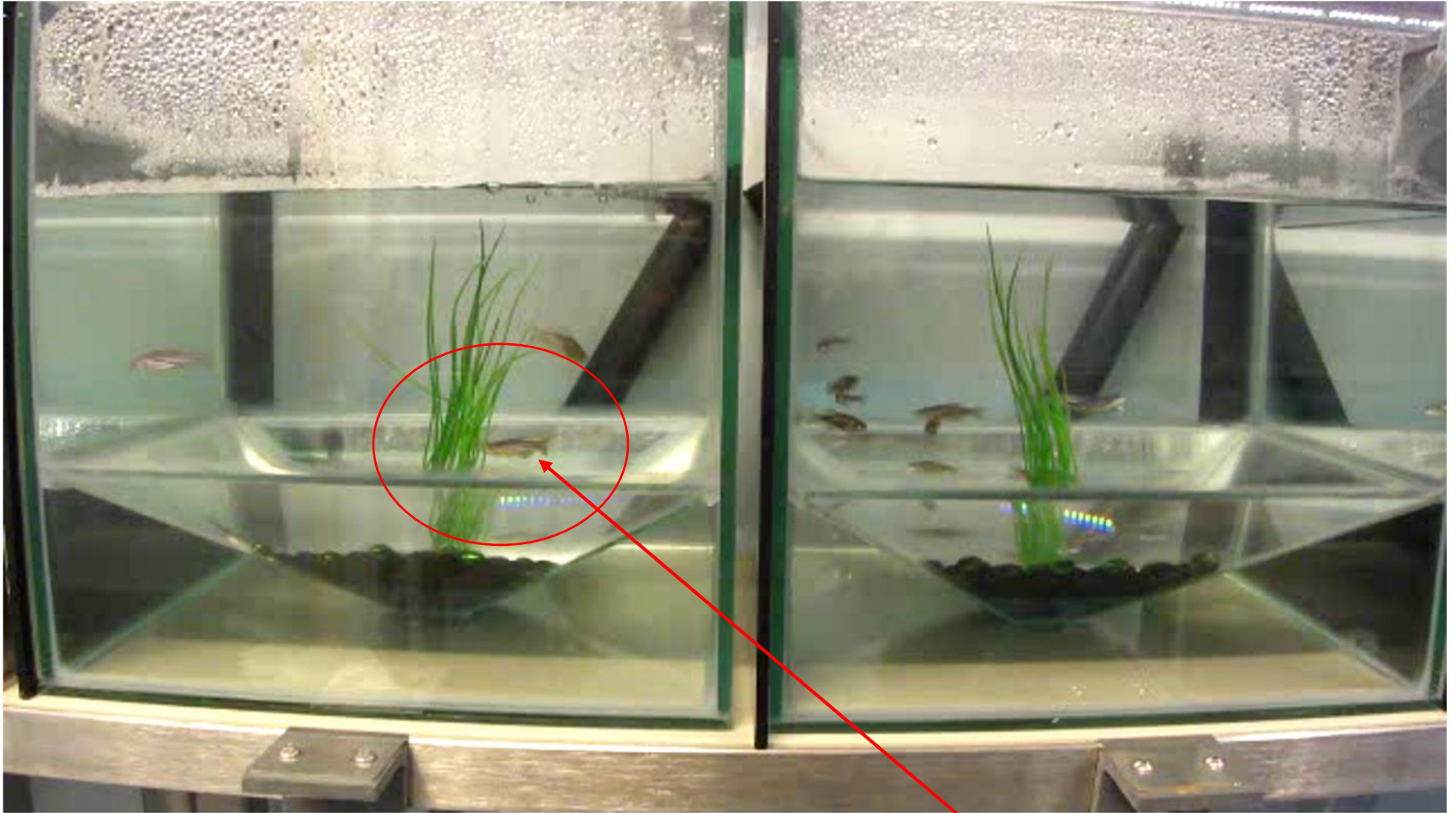


Paull et al., 2010. Dominance hierarchies in zebrafish and their relationship with reproductive success

Sexing Zebrafish



Sexing Zebrafish



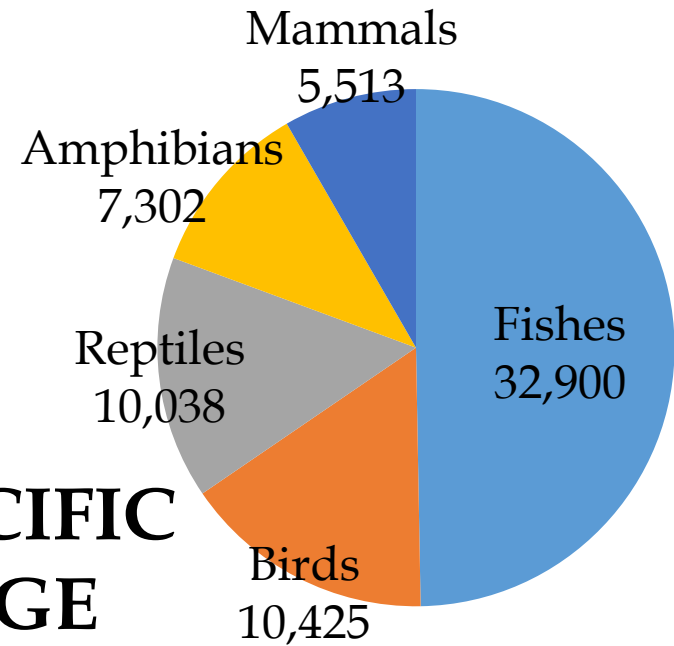
Territorial behaviour good
behavioural trait to ID males

- Behavioural characteristics to look for when sexing your fish
 - ▣ Of course you may observe courtship displays making sexing easy....but too late!
 - ▣ Males circling females, swim back and forth from the female to the selected spawning site 'ushering' the female to join, males also exhibit quivering motions, and nudging/biting behaviour around urogenital pore
 - ▣ During these courtship displays the dominant male will be very territorial often occupying and defending the central area of the tank, or preferred spawning site

- Behavioural characteristics to look for when sexing your fish
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 - ❑ During these courtship displays the dominant male will be very territorial often occupying and defending the central area of the tank, or preferred spawning site
 - ❑ Take advantage of both morphological and behavioural traits for sexing fish

EASY TO OBSERVE
IN YOUR TANKS!

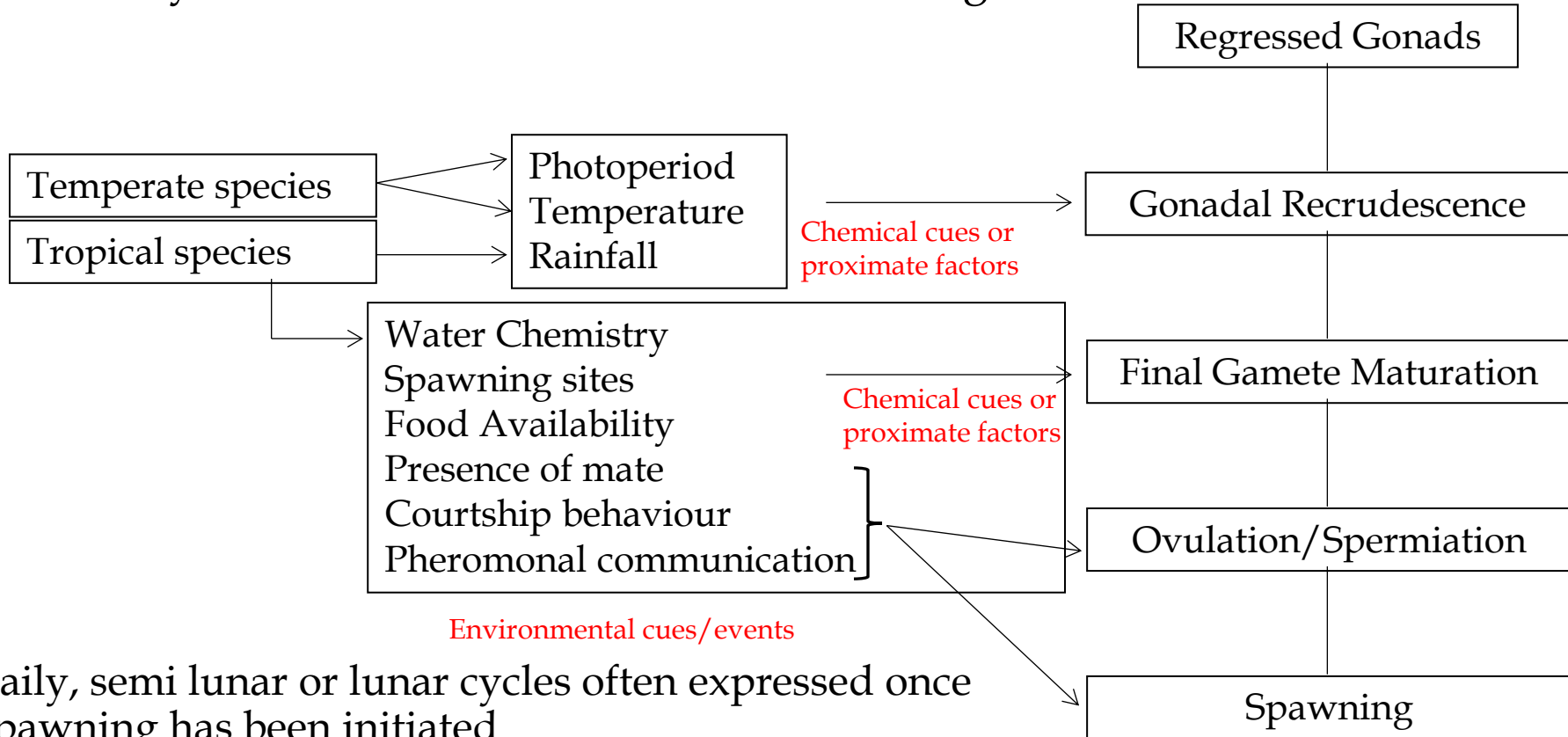
SPECIES SPECIFIC KNOWLEDGE



- a. making the right choices – improve reproductive output
- b. management opportunities – easier for you!
- c. inform on best welfare practices

Species Specific Knowledge

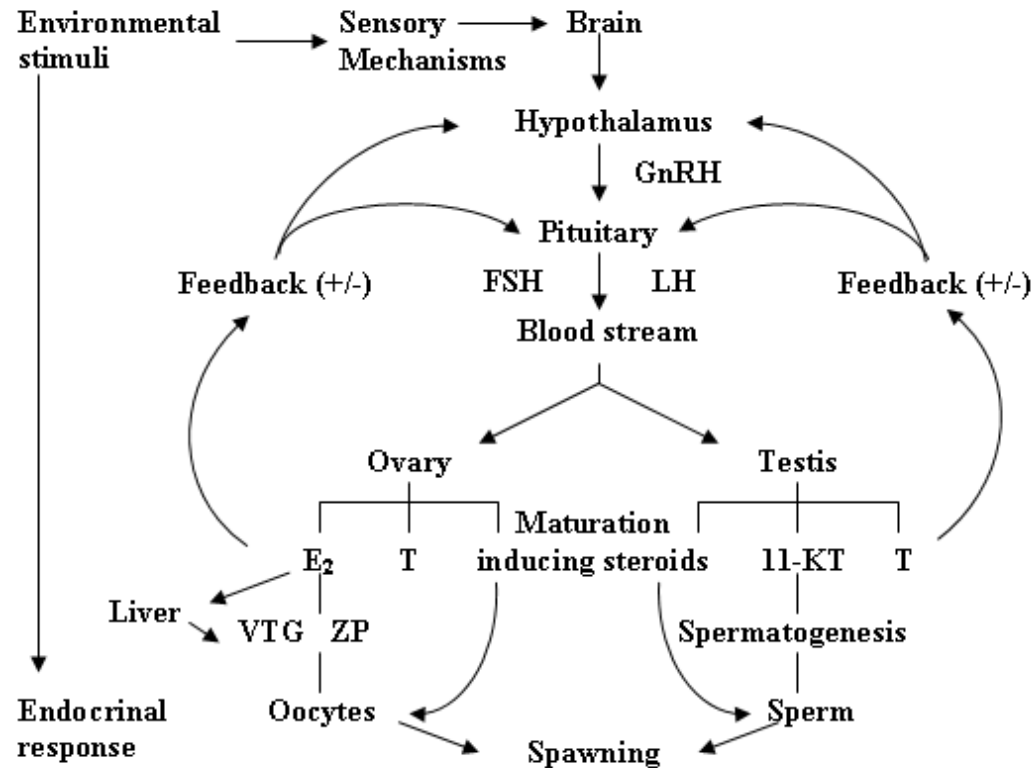
Successful sexual reproduction – species must synchronise gamete maturity with behaviour within and between genders



- daily, semi lunar or lunar cycles often expressed once spawning has been initiated
- spawning may not be cued by season alone?

Stimulate Reproductive status
Reproductive cycle of teleost fish
(adapted from Kime, 1998)

Species Specific Knowledge



The Brain-Pituitary-Gonadal axis (BPG) – gametogenesis to development of secondary sex characteristics, to behaviour

GnRH - gonadotrophin releasing hormone; FSH - follicle stimulating hormone; LH - luteinising hormone; E₂ - estradiol; T - testosterone; 11-KT - 11-ketotestosterone; VTG - vitellogenin; ZP - zona pellucida; (+/-) - positive/negative feedback. Adapted from Kime (1998)

Species Specific Knowledge – Wild studies



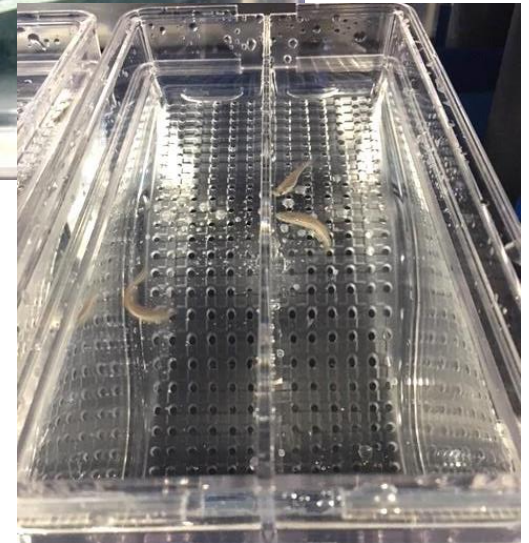
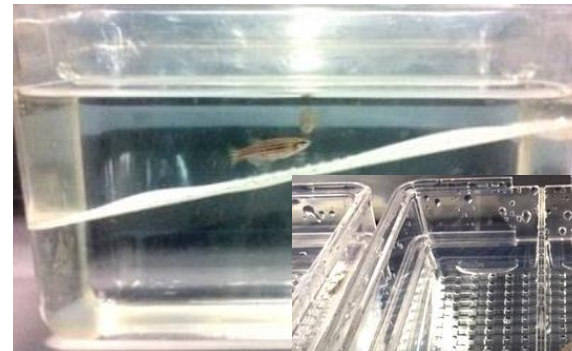
- Our knowledge is based on studies conducted within tanks in situ:
 - Spence & Smith published a series of papers on zebrafish ecology
- Observed similar reproductive behaviour to domesticated strains
 - males actively pursuing females up & down the water column before diving to the substratum to spawn; also males defending spawning sites
 - group spawning, egg scatterers, no parental care
 - both sexes shown to be choosy with respect to their oviposition site
 - spawning typically observed during the first hours of daylight & regular spawning cycles expressed once spawning initiated
 - spawning also observed at other times of the day following heavy rainfall

Species Specific Knowledge – lab studies

The reproductive behaviour of zebrafish is complex and has been shown to be effected by a wide range of variables including: light cycle, diet (Markovich 2007), health (Matthews 2004, Ramsay 2009b), stress (Ramsay 2009a) the age of the fish (Eaton and Roger 1974), the size of the fish (Paul et al., 2008, Spence & Smith 2006, Uusi-Heikkila 2010), dominance behaviour (Gerlach 2006, Paull 2010, Spence & Smith 2006), a female's preference for a male (Darrow & Harris 2004; Spence and Smith 2006), phenotypic traits, (Pritchard 2001) kin recognition (Gerlach & Lysiak 2006), females 'withholding' eggs for future mating events (Vaughan 2012, Skinner 2009), the interval since the fish last spawned (Eaton and Roger 1974, Niimi and LaHam 1974), stocking density, (Ramsay 2006, Goolish 1998, Spence and Smith 2005), sex ratio (Spence and Smith 2005, Ruhl 2009) if the fish have been sex isolated, oviposition site (Basquil 1998, Spence 2007), breeding chamber design (Engeszer 2007, Spence 2007, Sessa 2008) and the presence or absence of pheromones in the water (Gerlach 2006, van de Hurk 1992, Chen 1975).

Species Specific Knowledge

How do we incorporate this breeding information into breeding methodologies to enhance spawning output



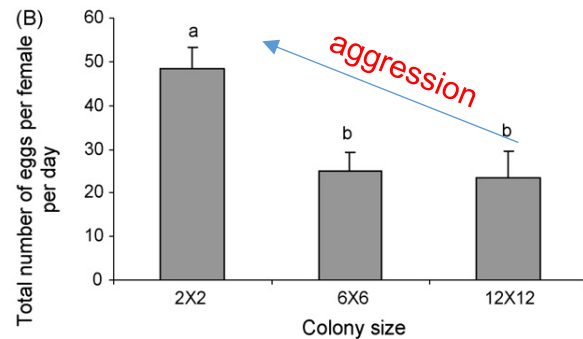
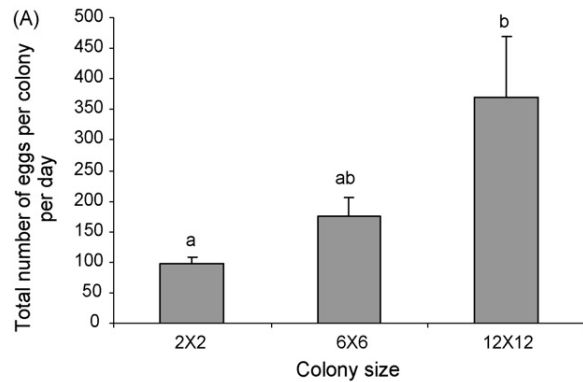
7th Annual International Zebrafish
Husbandry Meeting

Species Specific Knowledge

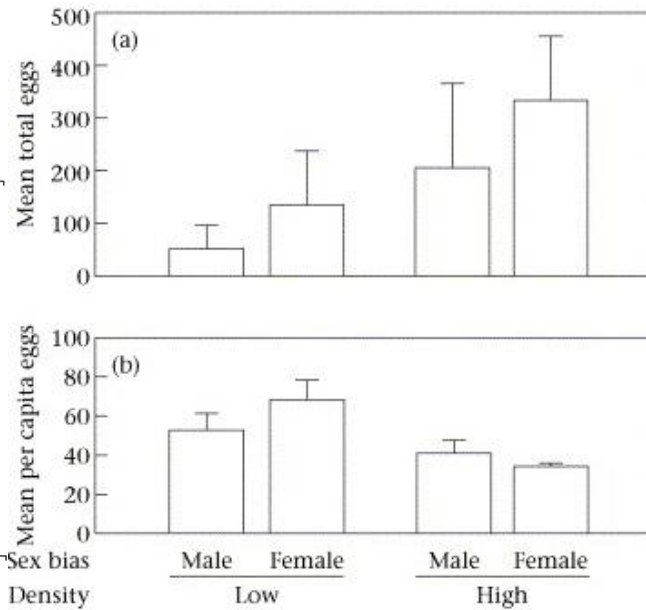
- My fish aren't spawning?
- What are your drivers? What are you trying to do?
- How are they set up?
 - Within tank
 - Within Chamber
 - Type of chamber
 - Groups; pairs; 2:1; 1:2
 - Have I got the right sex/sex ratio?
- When were they last spawned?
- How old are they?
- Are they in good condition?
- Are they being fed enough?
- Are the water conditions okay?
- Are the fish right for each other?
 - You chose the fish - they didn't choose each other

Species Specific Knowledge

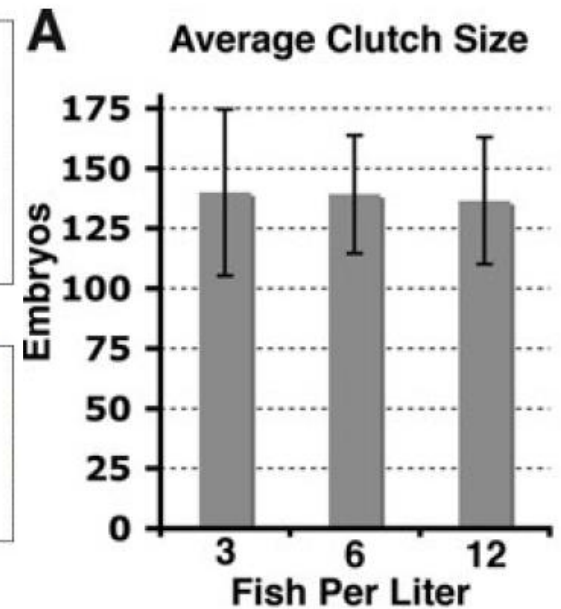
- Does density matter for reproductive success?



Paull et al., 2008. Aquatic Toxicology (87) 115-126



Spence & Smith., 2005. Animal Behaviour (69) 1317-1323



Castanova et al., 2011. The effect of stocking densities on reproductive performance in laboratory zebrafish

0.25 fish/L ←————→ 12 fish/L

Species Specific Knowledge

- Resource?

Table 1. Comparison between conventional crosses and breeding vessel.

	Conventional Crosses (40)	Breeding Vessel (1)
Step	Average Time (minutes)	
Setup (day before)	77±6	22±2
Setup (morning of)	13±3	2±1
Breakdown	5±1	2±1
Embryo Collection	27±6	2±0.6
Total time	122±7.6	29±2.6
Space required (ft ²)	16.7	2.92
Total embryos produced	4234±212 ^a	8400±794 ^b
Embryo viability (proportion)	0.87±0.02 ^a	0.86±0.006 ^a

Adatto et al., 2011. A New System for the Rapid Collection of Large Numbers of Developmentally Staged Zebrafish Embryos

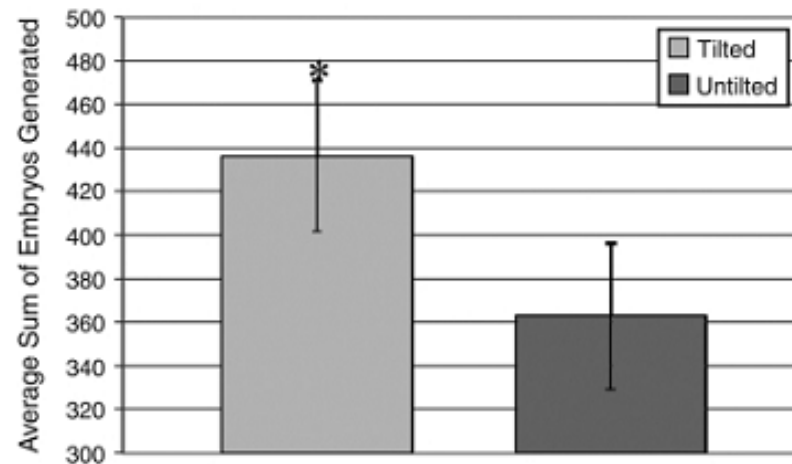


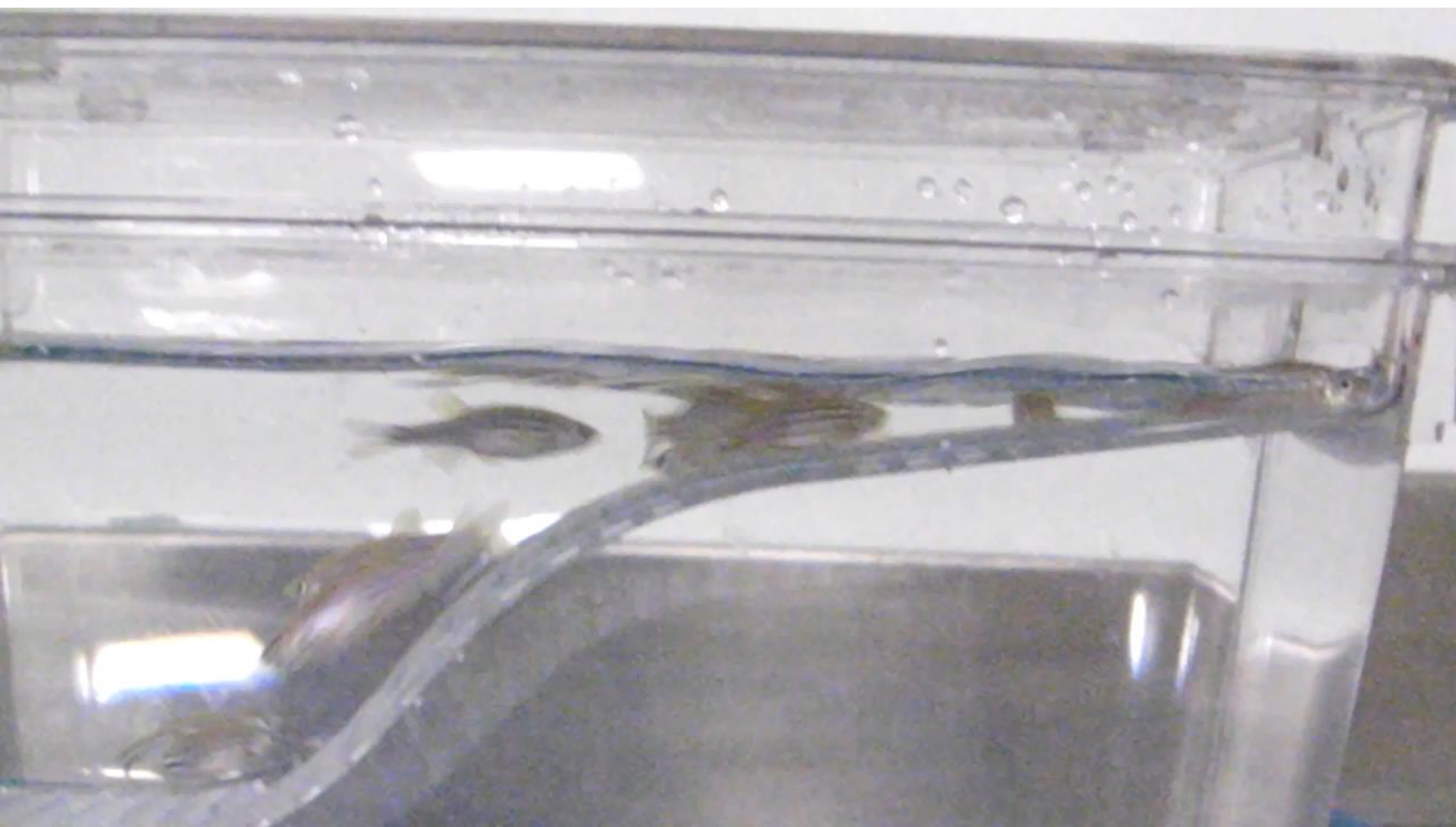
Species Specific Knowledge

- Does your spawning set-up matter for reproductive success?
- Preference for shallow water



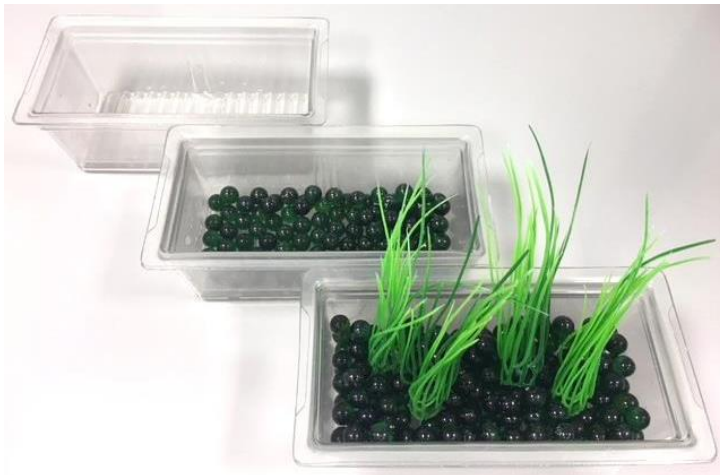
Sessa et al., The Effect of a Depth Gradient on the Mating Behavior, Oviposition Site Preference, and Embryo Production in the Zebrafish, *Danio rerio*. *Zebrafish*. 2008 December; 5(4): 335-339





Species Specific Knowledge

- Does your spawning set-up matter for reproductive success?



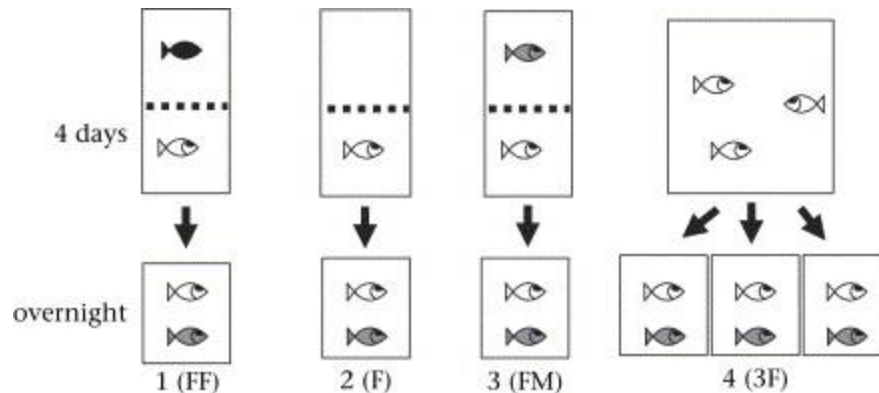
Zebrafish shown to be choosy with respect to their oviposition site

Species Specific Knowledge

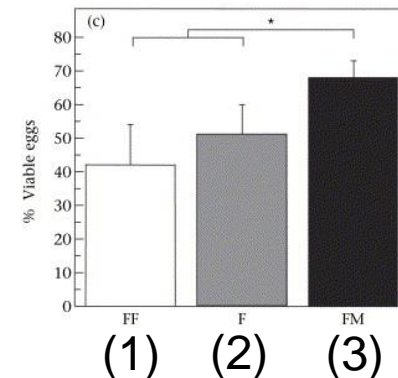
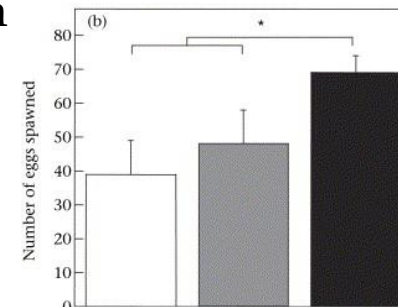
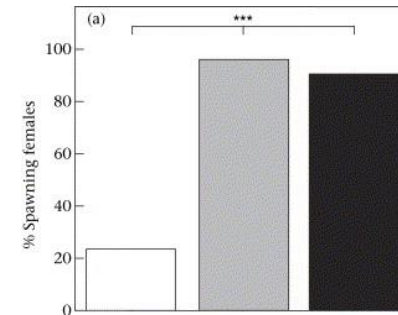
- Are olfactory cues important for reproductive success?

- Evidence that male fish release a primer pheromone to trigger female ovulation in a number of teleost species (Peter & Yu, 1997)

- Exposure to male pheromones can increase both egg output and egg viability in zebrafish (Gerlach, 2006)



(4) Dominant females spawned more viable eggs when placed with a male

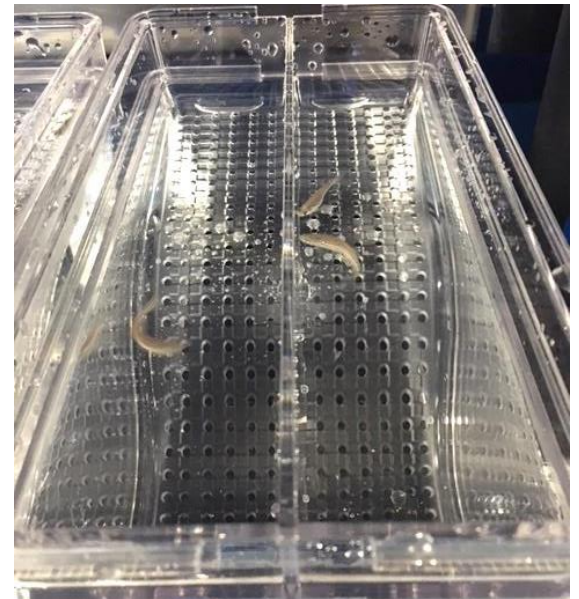
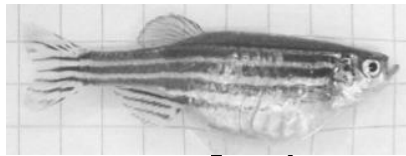


Species Specific Knowledge

- ▣ Are olfactory cues important for reproductive success?
- ▣ Reproductive suppression of female zebrafish kept together for four days prior to mating (Delaney et al., 2002)
 - females held in single sex groups for 4 days prior to mating laid only 45 +/- 12 (SE) eggs vs 200 +/- 12 (SE) in isolated females which were then mated

Species Specific Knowledge

- Does your spawning set-up matter for reproductive success?
- Use of dividers allows water flow between the sexes
- Housing fish separately has negative effects on egg output
- Maintaining mixed sex populations important for reproductive success in the long term



Species Specific Knowledge



- Does spawning frequency matter for reproductive success?
- Zebrafish can spawn on a near daily basis
 - Spence & Smith (2005) - females are capable of spawning at least 12 days in succession
 - large variation in egg numbers – consistent, cyclical, intermittent, irregular
- Clutch size is positively correlated with inter-spawning interval
 - inter-spawning interval ranged from 1-6 days producing clutches from a few eggs to several hundred (Spence & Smith, 2006)
- Females that are not spawned regularly (left in female biased colonies or isolated without male interaction) more susceptible to becoming ‘egg-bound’
- Evidence suggests useful to rotate spawning regularly within that 6 day cycle
 - Understanding often required on a line by line basis

Species Specific Knowledge

- ❑ Does size matter for reproductive success?
- ❑ Minimum body size at which fish are mature & are able to spawn
 - Female gamete production related to body size, not in males
- ❑ Body size rather than sex has been shown to be a predictor of dominance rank in many fish species
- ❑ Including in zebrafish (Grant & Kramer 1992; Hamilton & Dill 2002; Pyron, 2003; Spence & Smith 2005 Paull et al., 2010)
- ❑ Large dominant individuals have access to the best resources
 - spawning sites, food, shelter
- ❑ Potentially more attractive to the opposite sex

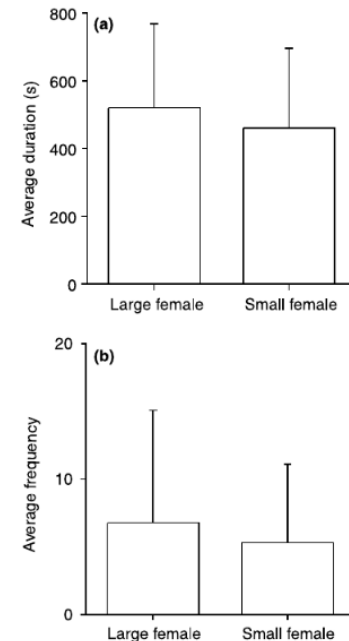
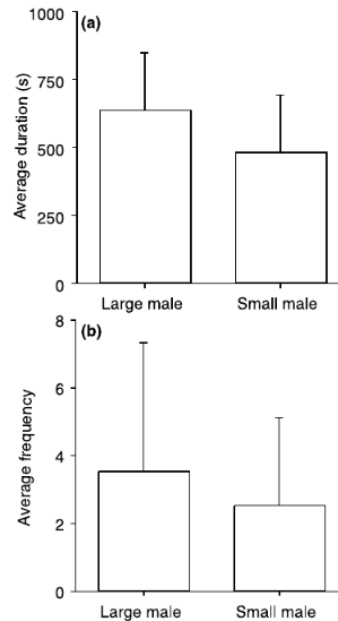
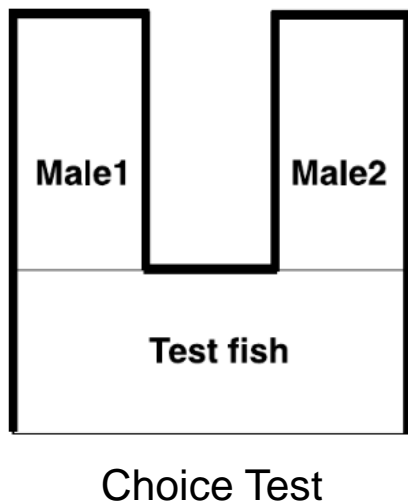


Atlantic silverside
(*Menidia menidia*)

Species Specific Knowledge

- Does size matter for reproductive success?

- redlip blennies shown to release more eggs when spawning with larger males (Cote & Hunt., 1989)
- no such correlation was observed in zebrafish in a study by Spence & Smith, 2006

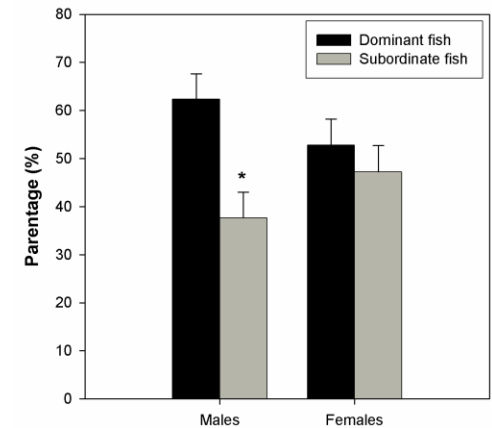
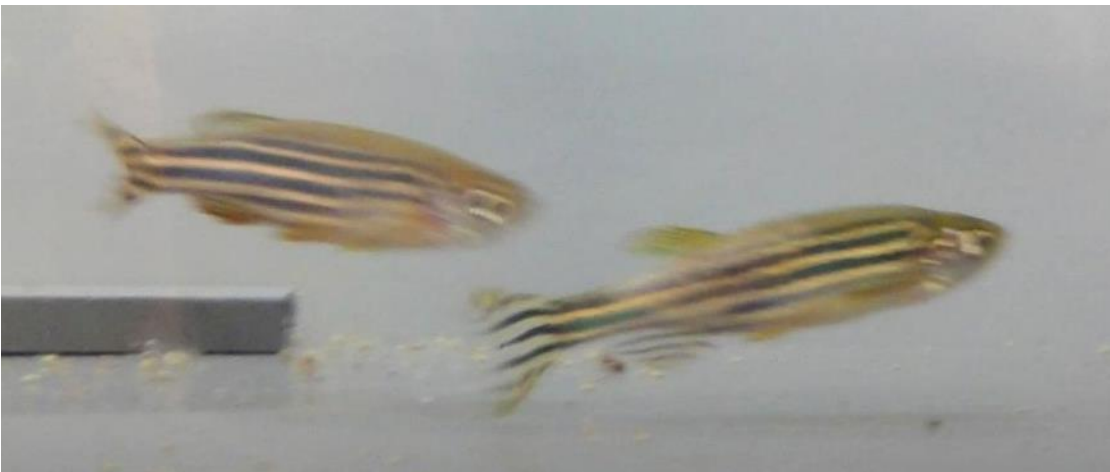


Pyron M. female preferences and male-male interactions in zebrafish (Danio rerio). Can. J. Zool 2003 (81):122-125.

- female spent more time with larger males whilst males showed no preference for females

Species Specific Knowledge

- Does size matter for reproductive success?
- Interestingly, parentage analysis in controlled laboratory experiments revealed:
- larger dominant males enjoyed greater reproductive success
- dominant females enjoyed greater reproductive success with the larger dominant male



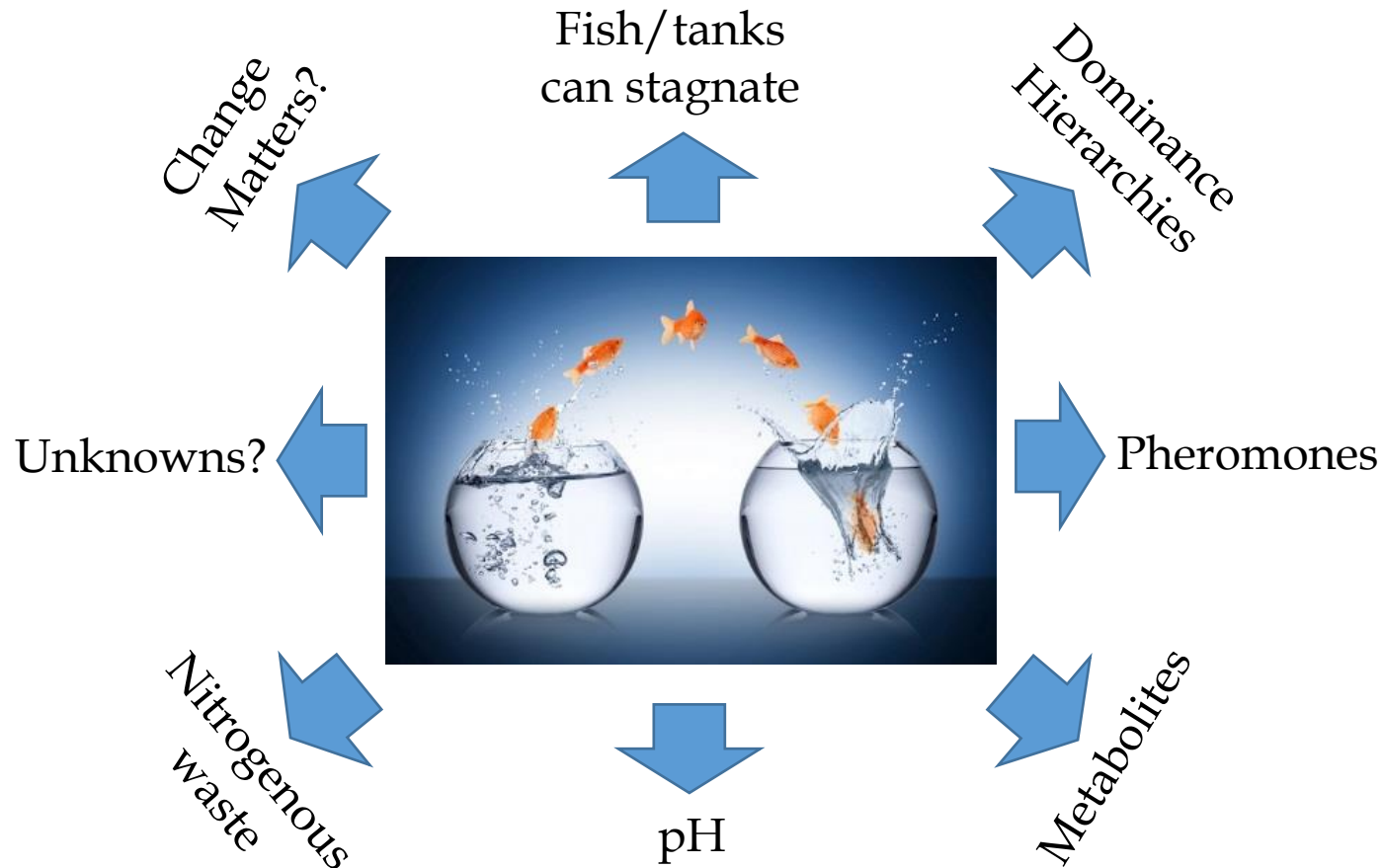
Paull GC, et al., **Dominance hierarchies in zebrafish (*Danio rerio*) and their relationship with reproductive success.** *Zebrafish* 2010 Mar;7(1):109-17.



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Species Specific Knowledge

Are water conditions important for reproductive success?



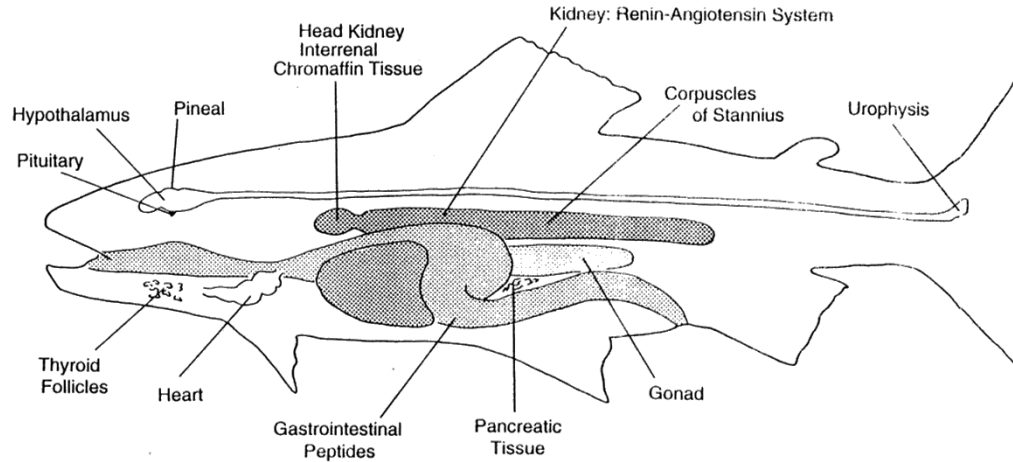
Spawning and Reproduction – Conclusion

- Better understanding of your study species is critical....
 - For you – saving you time and energy
 - when requests come in you can make quicker and more informed decisions
 - helps with problem solving - we all have difficult lines to spawn
 - For keeping research programmes on track
 - ensures appropriate delivery of embryos/other life-stages
 - timing, quality and number
 - For the welfare of your fish
 - life-experience in your facility
 - balance fish behavior & egg output
 - better welfare means better science!



Species Specific Knowledge

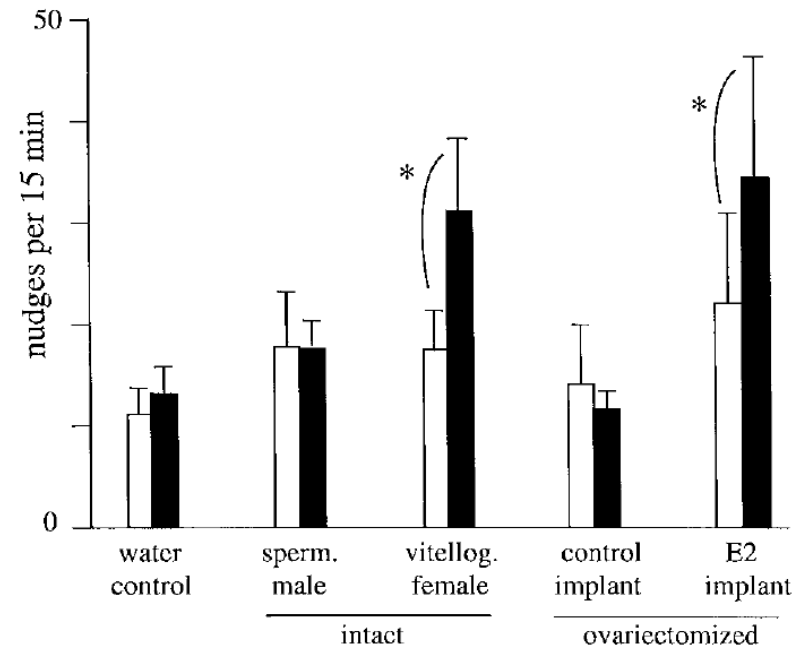
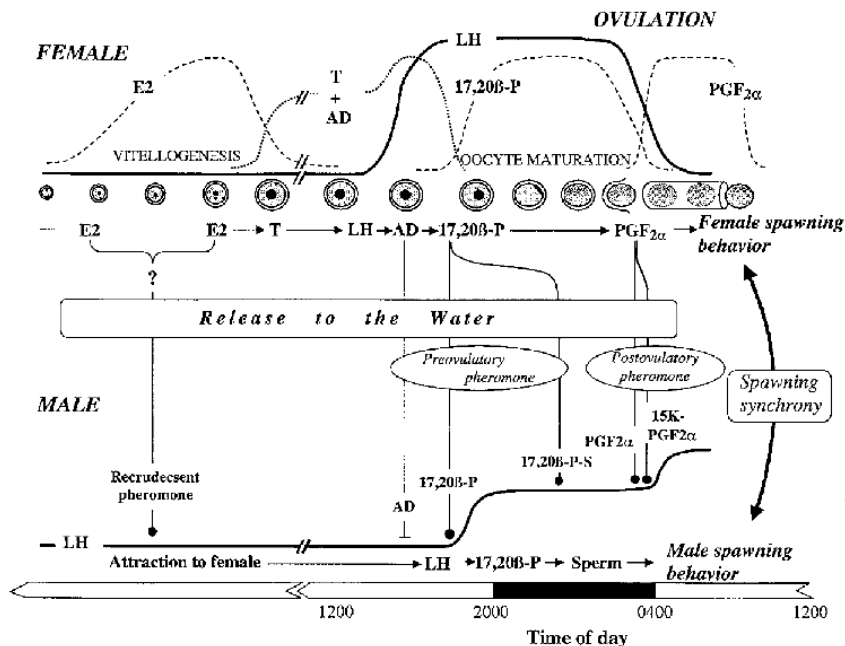
Reproduction in teleost fish is a complex process



- Primarily controlled by the endocrine system
- Regulated by the brain via a number of endocrine glands/tissues located throughout the body
- Hormones serve as the chemical messengers between these glands and their target tissues
- Regulates changes in expression of specific target genes culminating in physiological effects

Species Specific Knowledge

- Are Olfactory cues important for reproductive success?
- Evidence that female pheromones increase sperm quality and mobility in goldfish (Defraipont & Sorensen, 1993)



E2 (17 β -estradiol); T (testosterone); LH (luteinizing hormone); AD (androstenedione); 17,20B-P (17,20 β -dihydroxy-4-pregnen-3-one), PGF₂ α (prostaglandin F₂ α) & metabolite 15K-PGF₂ α