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Regeneráció és öregedés édesvízi hidráknál: életmenet döntések és fiziológiai hátterük

Regeneration and senescence in freshwater hydras: life history decisions and their physiological background

Egyetemi doktori (PhD) értekezés

szerző: Sebestyén Flóra Zsuzsa
témavezető: Dr. Tökölyi Jácint

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Nyilatkozom arról, hogy a tézisekben leírt eredmények nem képezik más PhD disszertáció részét.
Debrecen, 2021

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Sebestyén Flóra Zsuzsa

Tanúsítom, hogy Sebestyén Flóra Zsuzsa doktorjelölt 2017- 2021 között a fent megnevezett Doktori Iskola Evolúció- és diverzitásbiológia programjának keretében irányításommal végezte munkáját. Az értekezésben foglalt eredményekhez a jelölt önálló alkotó tevékenységével meghatározóan hozzájárult. Nyilatkozom továbbá arról, hogy a tézisekben leírt eredmények nem képezik más PhD disszertáció részét.
Az értekezés elfogadását javasolom.

Debrecen, 2021 ………………………….
Dr. Tökölyi Jácint

Regeneráció és öregedés édesvízi hidráknál: életmenet döntések és fiziológiai hátterük

Értekezés a doktori (Ph.D.) fokozat megszerzése érdekében
a ..Biológia... tudományágban

Írta: ..Sebestyén Flóra Zsuzsa..... okleveles .....biológus.......

Készült a Debreceni Egyetem ..Juhász-Nagy Pál................ doktori iskolája
(...Evolúció- és diverzitásbiológia...programja) keretében

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Tartalomjegyzék / Contents

> “If there were no regeneration there would be no life. If everything regenerated there would be no death. All organisms exist between these two extremes.”
>
> Richard Goss (1969)

1. Regeneráció és öregedés édesvízi hidráknál: életmenet döntések és fiziológiai hátterük

1.1. Bevezetés:

1.1.1. A regeneráció fogalma, őssejtekkel és ivartalan szaporodással való kapcsolata

A többsejtű élőlények számára állandó veszélyt jelentenek a sérülések, környezeti faktorok, betegségek vagy a stressz. A keletkezett károsodással szembeni védekezésként két fő folyamat alakult ki az evolúció során: a sebképződés és a regeneráció (Stocum, 2018). A sebképződés az elvesztett testrész pótlása nélkül történik, és a funkcionalitás csökkentése révén az adott terület integritásának megőrzésére szolgál (Stocum, 2018). Ezzel szemben, a **regeneráció** a negatív hatások helyreállító folyamatait foglalja magába (Stocum, 2018). Az általánosabban elterjedt definíció szerint a regeneráció az elveszett testrészek pótlását jelenti, amely vonatkozhat sejt, szövet, szerv vagy akár teljes test regenerációjára is (Bely & Nyberg, 2010).

A regeneráció tudományos történetének főbb pontjai betekintést adnak fogalmának alakulására is. A regenerációs kutatások kezdete a 18. századra nyúlik vissza, amikor is René-Antoine Ferchault de Réaumur 1712-ben elsőként végzett kísérleteket édesvízi rákokon, mely során végtagamputációt követő regenerálódásra irányultak megfigyelései (Ratcliff, 2012). Réaumur részletes, sikeres láb és olló regenerációt bemutató leírásának hatására a tudományos érdeklődés felerősödött a regeneráció jelensége iránt. A kor egyik legmeghatározóbb kutatása Abraham Trembleynek tulajdonítható, aki Réamur módszereit továbbfejlesztve hidrákon kísérletezett (Esposito, 2013), vizsgálataival megteremtve a kísérleti és hipotézis tesztelő biológia alapjait (Dinsmore 1991). A gerinceseken végzett részletes regenerációs vizsgálatok megkezdése a kor harmadik jelentős alakjának, az ebihalakat vizsgáló Lazzaro Spallanzaninek volt köszönhető a 18. század végén (Capanna, 1999). A 19-20. században azonban a regeneráció fogalma különböző pontosítási törekvések által vitatottá vált és két nézet alakult ki. August Weismann (1893) a regeneráció fogalmát egyszerűen a fiziológiai sejtmegújulás folyamataira alapozta, míg Thomas Hunt Morgan (1901) empirikus vizsgálatai alapján komplexebbnek tekintette a definíciót és két fő típust különböztetett meg. Kategorizálása szerint a sejtosztódást és az osztódó sejtekből történő blastema formálódást igénylő regenerációt *epimorfózis*nak (blastema: a sérülés helyén kialakuló differenciálatlan sejtek halmaza, mely létrehozza a regenerálódó testrészt (Bely and Nyberg, 2010)), a szöveti átrendeződés révén megvalósuló regenerációt *morphallaxis*nak nevezte (Morgan, 1901). Az epimorfózisnak további három esetét is elkülönítette aszerint, hogy az újonnan létrehozott szerv megegyezett az előzővel (‘homomorphosis’), kisebb volt a helyettesített szervhez képest (‘meromorphosis’) vagy morfológiailag és fiziológiailag is eltért a korábbitól (‘heteromorphosis’) (Esposito, 2013). A fogalom körüli értekezések a kor nézeteit is jól tükrözték. Míg Weismann szerint az állati regeneráció kialakulása esetleges, és természetes szelekció eredményezte adaptációnak köszönhető, addig Morgan ragaszkodott hozzá, hogy egyik állítás sem igaz és minden élőlény univerzálisan meglévő, különböző mértékben megjelenő tulajdonságát jelenti (Esposito, 2013). Habár mindkét definíció empirikusan, más kutatók munkája által is alátámasztható volt (Esposito, 2013), a vita eldöntését megnehezítette az akkor még nem ismert mai korszerű vizsgálati technológiák hiánya is.

A folyamatos kutatás ellenére tudásunk továbbra sem teljes, így a kérdésre, hogy a regeneráció adaptív vagy eredendően meglévő jelleg-e, oly sok más kérdéssel együtt, a mai napig sem tudunk minden kétséget kizáróan választ adni. Az eddigi eredmények azonban azt sugallják, hogy a regeneráció a valódi szövetes állatok egyik alapvető, embrionális fejlődéshez kapcsolódó jellege, elvesztése pedig más, fittness szempontjából előnyösebb jelleg megjelenéséhez kapcsolható, pleiotropikus jelenség következményeként (Goss, 1992). Újabb kutatás szerint, egyes testrészek regenerációjának evolúciós kialakulására is van bizonyíték (Zattara et al., 2019) A jelenség összetettsége is hozzájárulhatott ahhoz, hogy a regeneráció fogalma egyre specifikusabb helyett egyre inkább széles körben kiterjedővé vált több mint 300 éves kutatási múltja alatt. **Jelenleg regenerációként elfogadottnak tekintett számos helyreállító folyamat, melyek kifejeződése akár fiziológiai ciklikusságnak (például vér és bőr megújulása), akár sérülésnek köszönhető, továbbá a regeneráció orvoslási aspektussal, a „regeneratív orvoslás" ággal is kibővült** (Sánchez Alvarado, 2012).

A regeneráció intenzív kutatásának köszönhetően régóta használatos fogalmak is pontosíthatók és új, általános érvényű mintázatok is felfedezhetőek. Morgan epimorfózis és morphallaxis fogalom meghatározásai munkássága után is igen elterjedtek maradtak, ám a két típus klasszikus elkülönítése a mai napig vitatott (Agata et al., 2007; Kostyuchenko & Kozin, 2020; Maden, 1981). A két kategória egyértelmű elkülönítési problémáinak demonstrálására jó példa a planáriák regenerációja. A planáriákon ejtett vágás után szabad szemmel is észrevehető, az epimorfózisra jellemző blastema formálódás figyelhető meg, ám morphallaxisnak tekintett folyamat, a csonk szöveti átrendeződése is lezajlik. Már maga Morgan is leírta mindkét jelenséget, de később is igazolták, hogy mind epimorfózisra, mind morphallaxisra jellemző folyamatok lejátszódnak esetükben (Rink, 2018). A két fogalom fő, sejtosztódással kapcsolatos kritériumai is egyértelműen megdőlni látszanak: az epimorfózis során a sejtosztódás nem jár mindig a blastema kialakulásával, továbbá morphallaxis során is játszódhat le sejtosztódási folyamat (Pellettieri, 2019). A molekuláris és sejt szintű vizsgálatok fejlődése és térnyerése révén az újabb eredmények azt is alátámasztják, hogy a szöveti átrendeződés (vagyis morphallaxis) az epimorfótikus regeneráció inicializáiójához, köztes stádiumaihoz és befejezéséhez is szükséges gyűrűs férgek esetében (Kostyuchenko & Kozin, 2020). A két regenerációs típus nehéz besorolása miatt a szakirodalomban potenciálisan zavarosság fedezhető fel (Agata et al. 2003), mely hidrák esetében is igaz (Chera et al., 2009). Ezért disszertációmban sem hivatkozom rájuk.

A regeneráció kutatás fejlődése azonban nem csak régóta használt fogalmak újragondolását tette lehetővé, hanem rávilágított új, széles körű tendenciák jelenlétére is, mint például az **őssejtek** szerepére (Gurley & Alvarado, 2019). Az őssejtek jelentős közreműködése az állati regenerációban széles körben ismert, legyen szó szivacsokról, laposférgekről vagy emlősökről (Funayama, 2018; Gurley & Alvarado, 2019). Az őssejtek olyan sejtek, melyek folyamatosan megújulnak és új, differenciálódott sejtek létrehozására képesek (Adler & Alvarado, 2015). Jellemzően nyugalmi állapotban vannak vagy lassú sejtciklussal rendelkeznek, ám sérülés hatására számos funkciójuk aktiválódhat (Bergmann & Steller, 2010). Az új sejtek létrehozásával a sérülés okozta szöveti veszteség pótlásának egyik fő forrását jelenthetik (más sejttípusok de-, re- és transzdifferenciációja mellett (Sugimoto et al., 2011)), részt vehetnek a blastema kialakításában és számos jelátviteli út indukálásában (Gurley & Alvarado, 2019). Egyes szivacs fajoknál őssejtjeik, azaz az archeociták részt vesznek a blastema szerű struktúra kialakításában és transzdifferenciálódásában (Funayama, 2018). *Drosophila* esetében a bélőssejtek kompenzációs osztódásuk révén lehetővé teszik a teljes középbél szakasz regenerációját 2-3 nap alatt (Bergmann & Steller, 2010). Planáriákban az őssejtek (esetükben neoblastok) osztódása a testben az amputációt követően 6 órával megemelkedik, 48 óra elteltével pedig az osztódó őssejtek már a sérülés helyén találhatóak meg, sőt akár a teljes test regenerációját is lehetővé teszik (Ricci & Srivastava, 2018). Gerincesek izomszöveteire jellemző, hogy őssejteket (szatellit sejtek) tartalmaznak, melyek osztódása sérülés hatására aktiválódik, majd pedig differenciálódást követően az izomrostok helyreállításában vesznek részt (Tanaka & Reddien, 2011). Emlősöknél a regeneráció elsősorban szövet specifikus őssejtektől függ (Yun, 2015), fontosságuk az ember esetében is ismert és aktívan kutatott (Carlson, 2011). Habár a vizsgált szervezeteknél az őssejtek eltérő kontextusban jelenhetnek meg, szerepük számos esetben kritikus a regeneráció létrejöttéhez (Gurley & Alvarado, 2019).

A regeneráció egy másik jelenséggel, az **ivartalan szaporodás**sal is szorosan összefügg. Egyes teóriák szerint a regenerációnak egy lehetséges evolúciós eredete az ivartalan szaporodásra vezethető vissza (Alvarado 2000). Ha a hasadással történő szaporodást vizsgáljuk, a csillós *Bursaria* esetében például a hasadása és regenerációja között a különbség egyedül a kiváltó stimulusban van: a hasadáshoz kedvező körülmények szükségesek, míg a regenerációt valamilyen sérülés váltja ki (Alvarado, 2000). A folyamat eleji dedifferenciáció, majd a két sejt darab felnőtt egyeddé történő redifferenciálódása regeneráció és hasadás során ugyanúgy zajlik (Alvarado, 2000). Gyűrűsférgeken végzett vizsgálatok azt igazolták, hogy a regenerációban és az ivartalan szaporodásban résztvevő epitóp és fehérje mintázatnak vannak közös résztvevői (Martinez et al., 2005), továbbá egy evolúciós modell is alátámasztja esetükben a közös evolválódás valószínűségét (Zattara & Bely, 2016). Hidráknál a fejregeneráció és a bimbózás során aktiválódó jelátviteli út is azonosnak bizonyult (Javois et al., 1986). A regeneráció és az ivartalan szaporodás számos állatban mutatott parallel mintázatai arra engednek következtetni, hogy a regeneráció evolúciós kialakulása az ivartalan szaporodási mechanizmusok sérülések során történő megjelenésével kezdődhetett (Alvarado, 2000). A hasonlóságok ellenére Bely és Nyberg (2010) úgy vélik, az egyedi kiváltó stimulus, egyes regeneráció specifikus fejlődési tulajdonságok megléte (például sajátos génexpressziós mintázatok) valamint az ivartalan szaporodással nem teljesen megegyező filogenetikai megjelenés azt támasztják alá, hogy a regeneráció önálló evolúciós jelenségként kezelendő.

Érdekes, hogy az ivartalan szaporodást az őssejtek lehetséges evolúciós eredeteként is leírták már (Agata et al., 2006). A hasadás példájánál maradva, planáriáknál tudjuk, hogy a hasadás utáni hiányzó testrészek regenerálódása a pluripotens őssejteik igénybevételével történik (Agata et al., 2006). Az őssejtek megléte, ivartalan szaporodásban való részvétele és a nagy regenerációs képesség együttes megjelenése szivacsoktól egészen gerinchúrosokig megfigyelhető, így többek között ezen mintázat alapján is született az a feltételezés, miszerint az őssejtek az ivartalan szaporodás komponenseként evolválódtak

(Agata et al. 2006). Az ivartalan szaporodás filogenetikai megjelenését vizsgálva észrevehető, hogy valóban egy széles körben elterjedt, a galléros ostorosokig visszavezethető jelleg (Alvarado, 2000; Sköld et al., 2009). A vizsgált taxonok nagy részében totipotens vagy legalább pluripotens őssejtek vagy flexibilis felnőtt sejtek részt vesznek a folyamatban, mely elterjedés nagy sejti és szöveti plaszticitás meglétét tükrözi (Sköld et al., 2009). Felmerül azonban a kérdés, hogy hogyan is kapcsolódik egymáshoz a három tényező, a regeneráció, az őssejtek és az ivartalan szaporodás. Az ivartalan szaporodás magyarázza a regeneráció és az őssejtek közötti szoros összefüggést? A regeneráció nem egyeséges filogenetikai elterjedése azonban minek köszönhető? Mi lehet az, ami egyes fajokban és egyedekben gátolja vagy elősegíti a regeneráció képességét?

1.1.2. A regeneráció mint életmenet jelleg, költségei az őssejtek vonatkozásában

A tágabb, általános kérdések alapjaihoz és megválaszolásához közelebb vihet minket, ha a regenerációt mint *életmenet jelleget*, vagyis mint szaporodást és túlélést befolyásoló tulajdonságot vizsgáljuk. Ehhez fontos figyelembe venni, hogy a regeneráció egyértelmű előnyei mellett számos költséggel is rendelkezik. Ilyen költség lehet i) az átmeneti fázisok megléte (a még nem teljesen regenerálódott végtag nagyobb hátrányt jelent az állat számára, mintha az adott testrésze hiányozna), ii) a pontos regeneráció költsége (ha a regenerálódott testrész nem teljesen azonos, torz az eredetihez képest) vagy iii) a regeneráció energia- és forrásallokációs költsége (Bely & Nyberg, 2010). A regeneráció energia költsége több fajnál is kimutatott. *Liolaemus belli* gyíkoknál a farok regenerációja során 36%-al megnő a standard metabolikus rátájuk (Naya et al., 2007). *Gerrhonotus multicarinatus* gyíkok akár a teljes energiájuk több mint felét a farok regenerációjára fordíthatják (Vitt, Congdon & Dickson, 1977). *Parabuthus transvaalicus* skorpióknál 39%-os a metabolikus ráta emelkedés méreg készlet regeneráció során (Nisani et al., 2007). A tüskésbőrűeknél (tengeriliomok, kígyókarúak, tengericsillagok) pedig a kar regenerációja során a csökkent táplálékfelvételi képességből adódik a fő energia költség (Lawrence, 2010). A regeneráció fennálló energia- és forrásköltsége révén az élőlények számos más, életmenetükhöz tartozó jellegére is hatással lehet. Ez igen jelentős felismerés, ugyanis az egyed életmenetének a születésétől haláláig tartó időszakot tekintjük, mely magába foglalja azokat a kor- és állapotfüggő mintázatokat, amelyek az ivarérést, szaporodást, túlélést és halált határozzák meg (Flatt & Heyland, 2011). A regeneráció költségei tehát befolyásolhatják az egyedek élettartam alatti szaporodási sikerét, más életmenet jellegekre gyakorolt hatásai révén.

Az életmenet jellegek által felhasznált források limitáltak, így elkerülhetetlen közöttük a negatív kapcsoltság, azaz csereviszonyi kapcsolatok kialakulása (Flatt & Heyland, 2011). Limitáló tényezők lehetnek többek között a makro- és mikro tápanyagok (Catoni et al., 2008; Cotter et al., 2011; Zera & Zhao, 2006), metabolikus tartalékok (Ellers, 1995), de ezeken felül bármilyen több életmenet jelleg számára szükséges és véges anyag is a csereviszonyi kapcsolatok tárgyát képezhetik. A nagy szöveti plaszticitással rendelkező állatoknál (pl. szivacsok, korallok, csalánozók, laposférgek) az őssejt készlet is ilyen limitáló tényező lehet (Rinkevich, 1996). Ezen gerinctelen állatok teste nagy arányban tartalmaz őssejteket, melyek egyfelől fontosak a túlélésért felelős számos jelleg kialakításában, mint például a növekedésben, a táplálékszerzésre szolgáló csalánsejt képzésében, és a már ismertetett regenerációban játszott szerepük is jelentős (Gold &

Jacobs, 2013; Henry & Hart, 2005). Másfelől viszont, ugyanezen őssejtek bizonyítottan felelősek az ivaros és ivartalan szaporodásban résztvevő sejtek képzésében is (például szivacsoknál: Simpson, 1984; csalánozóknál: Gahan et al., 2016; planáriáknál: Newmark, Wang, & Chong, 2008). A fentebbi folyamatok közös, véges őssejt készlete alapján elméleti megfontolások szerint várható, hogy ha például a szaporodásba való befektetés intenzív, akkor annak fiziológiai költsége is megnő, aminek eredményeképp az őssejtek szomatikus funkciójú, többek között regenerációban résztvevő sejtekké való differenciálódási képessége csökken a fennálló csereviszonynak köszönhetően (Henry & Hart, 2005; Rinkevich, 1996).

A regeneráció csereviszonyi kapcsolatokban való részvtele egyértelmű, azonban számos aspektusa még kevéssé ismert. Tanulmányaimmal ezeknek a körét bővítem a regeneráció vizsgálata révén őssejt készlet, ivaros és ivartalan szaporodási módok és életkor függvényében, valamint az intenzív öregedési folyamatokkal járó ivaros szaporodás és túlélés közötti forrásallokációs kapcsolat tanulmányozásának segítségével.

1.1.3. Regenerációs különbségek eltérő szaporodási módokban

A szaporodás és az önfenntartás közötti csereviszonyt jelentősenbefolyásolhatja a szaporodás módja, mely lehet ivaros és ivartalan is. Habár az ivaros szaporodás változó környezet esetén olyan egyértelmű előnyökkel jár, mint a rekombinálódott genotípusú utód, olyan költségekkel is rendelkezik, mint a hímek produkciója és a genom hígulása (Lehtonen et al., 2012). Az ivartalan szaporodás során nem merülnek fel ezek a költségek, így bizonyos körülmények között evolúciósan előnyösebb lehet (Crow, 1994). Az utódok szaporodási értéke fontos szerepet játszhat abban, hogy mely szaporodási mód a sikeresebb, például annak köszönhetően, hogy az utódok túlélési esélyei eltérőek az adott körülmények között. Ha az ivaros és ivartalan utódok szaporodási értéke eltérő, várhatóan a befektetett forrás mennyisége is különbözik, mely gyakran tapasztalt jelenség az állatok között (pl. zsákállatok: (Yund et al., 1997), levéltetvek: (Nespolo et al., 2009), *Daphnia*: (Innes & Singleton, 2000), hidra: (Kaliszewicz, 2011)). Arról azonban sokkal kevesebbet tudunk, hogy a szaporodási módtól függő eltérő befektetett energia mennyiség milyen fiziológiai költségekben nyilvánul meg, valamint milyen hatással van az állatok regenerációs képességeire és önfenntartásért felelős jellegeire. Az őssejtek lehetséges mediátor szerepéhez kapcsolódó vizsgálatok pedig különösen hiányoznak a korábbi elméletek alátámasztásához, mely kérdéskör a 1. tanulmány egyik központi eleme.

1.1.4. Korfüggő változások

A limitált források mellett a regeneráció mértékét és más életmenet jellegekkel való csereviszonyát jelentősen meghatározhatja az életkor és életszakasz is (Fischer et al., 2014). A kor olyan, az organizmus belső állapotához tartozó faktor, amely korrelál a kondícióval, szaporodással és túléléssel (McNamara & Houston, 1996). Továbbá a korral történő információ felhalmozódás révén, bizonytalanul változó környezetben a kor nagyban segítheti a döntéshozást (Fischer et al., 2014). A szomatikus sérülések helyreállító képességéről, regenerációjáról általában elmondható, hogy mértéke fiatal korban magasabb, ivarérést követően csökken, majd szinte teljesen megszűnik a maximális várható élettartam vége előtt (Cichoń & Kozłowski, 2000). Tekintve, hogy a regeneráció a forrás limitáltság miatt kapcsoltságban állhat más jellegekkel is, szabályozása szempontjából érdemes megvizsgálnunk az ivaros és ivartalan szaporodási mintázatokat is. Véges és végtelen növekedéssel bíró szervezetekre is gyakran igaz, hogy fekunditásuk az életkorral nő, majd a véges növekedésű fajok többségénél a szaporodási öregedés során fekunditás csökkenés figyelhető meg (Clutton-Brock, 1984; Martin, 1995). Klonális vagy parciálisan klonális (vagyis ivaros és ivartalan szaporodásra is képes) állatok esetében azonban a korfüggő mintázatok sokkal bonyolultabbak lehetnek és eltérhetnek a nem klonális állatoknál tapasztaltaktól (Clutton-Brock, 1984; Engen & Saether, 1994; Stearns, 1992), ezért az önfenntartást is tartalmazó életmenet modellekbe való integrálásuk is komplikált (Glazier & Calow, 1992). A konvencionális életmenet predikciók szerint az élőlények kezdetben a növekedést priorizálják, majd a növekedés abbamaradása után megkezdik a szaporodást (Cichoń, 1997). Ezzel ellentétben a klonális állatoknál vizsgálati eredmények azt mutatják, hogy a korai életszakaszban történő szaporodás majd későbbi növekedés is lehetséges, ha az egyedek jó kondícióban vannak (Glazier & Calow, 1992). A parciálisan ivartalanul szaporodó állatoknál fontos életmenet döntés az ivartalanról ivaros szaporodásra való váltás is, melynek időzítése rugalmas és lehetséges kiváltó tényezője az ivartalan szaporodás külső környezeti tényezők általi gátoltsága (Burke & Bonduriansky, 2018; Gerber et al., 2018; Harvell & Grosberg, 1988).

Míg az ivaros és ivartalan szaporodás közötti elméleti különbségek jobban ismertek (összefoglalva például: Meirmans et al., 2012; Neiman et al., 2014), a szaporodási módokkal *párhuzamosan*, a regeneráció és más önfenntartásért felelős jellegekkevésbé kutatottak, különösen ezek korral bekövetkező változásai. Az ivaros és ivartalan szaporodási módok regeneráció tekintetében is eltérhetnek, melyre általában az jellemző, hogy az ivartalan egyedek gyorsabb regenerációs képességgel rendelkeznek (például új-zélandi mocsár csigák (*Potamopyrgus antipodarum*) (Krois et al., 2013) és harántfogúgőte-félékhez tartozó szalamandrák esetében (Saccucci et al., 2016)). Az is bizonyos, hogy lehetségesek szokatlan mintázatok a regenerációban az életkor változásával. Így például fiatal szivacsok regenerációja teljesen gátolt lehet (vélhetően az őssejtek intenzív növekedésben való részvétele miatt, (Simpson, 1984)), valamint egyes parciálisan ivartalan fajok regenerációs képessége az életkor előrehaladtával sem csökken (Bodnar & Coffman, 2016; Yun, 2015). Az ivartalan egyedekre általánosan magas önfenntartási jellegek jellemzőek (Sköld & Obst, 2011), ugyanakkor, az életkor előrehaladtával bekövetkező csökkenés is tapasztalható többek között a regenerációban (Meesters & Bak, 1995), túlélésben (Orive, 1995) és DNS-t javító enzimek (pl. telomeráz) aktivitásban is (Sköld et al., 2011). Ahogyan a fenti, néhol ellentmondásos példák is bizonyítják, a regeneráció életkorral történő változásait korántsem értjük teljesen, a változó szaporodási módok és a regeneráció (és más önfenntartásért felelős jellegek) közötti forrásallokációs mintázatokról pedig különösen keveset tudunk klonális és parciálisan klonális állatok esetében. Ehhez kapcsolódó ismereteink bővítésére szolgál a 2. tanulmány.

1.1.5. Az öregedés és a regeneráció kapcsolata

A regenerációs folyamatok széles köre kapcsolatban állhat nem csak a korral történő változásokkal, hanem az öregedés jelenségével is. Az evolúcióbiológusok többsége az öregedést a fiziológiai funkciók korfüggő vagy korral fokozatosan kifejeződő hanyatlásaként definiálja, mely a korspecifikus mortalitási ráta növekedéséhez (vagyis a túlélési ráta csökkenéséhez) és a korspecifikus szaporodási ráta csökkenéséhez vezet (Flatt 2012). Dolgozatomban eddig elsősorban a sérülés indukálta regenerációval kapcsolatos összefüggésekkel foglalkoztam, azonban Stocum (2018) leírása alapján a regeneráció egy másik, öregedéshez is kapcsolódó típusa, a *fiziológiás vagy fenntartó regeneráció* is meghatározható. A fiziológiás regeneráció során a régi sejtek újakkal történő helyettesítése (azaz a sejt „turnover”) az a homeosztatikus folyamat, mely fenntartja és biztosítja a szövetek normál szerkezetét és funkcióját (Stocum, 2018). Ez a kívülről láthatatlan folyamat az összes többsejtű élőlénynél megtalálható, például olyan formában mint az epidermisz, vér és gyomor sejtek cseréje (Kostyuchenko et al., 2016). A sérülés indukálta regeneráció mellett az öregedési mechanizmusok erre a fiziológiás regenerációra is hatással vannak.

Az öregedés fiziológiás regenerációs képességre gyakorolt hatása alapvetően a sejtosztódás szabályozására való negatív hatásának a következménye (Seifert & Voss, 2013). Az öregedésért felelős folyamatok széles skálája ismert, történhet többek között a növekvő DNS károsodásnak, telomer eróziónak, a génexpresszió és fehérje funkciók szabályozatlanságának, a sejt mátrix és sejtszervecskék diszrupciójának, a megromlott sejtek közötti kommunikációnak, az oxidatív stressznek és a felborult metabolizmusnak köszönhetően is (McHugh & Gil, 2018; Sousounis et al., 2014). Ezen folyamatok megannyi módon képesek fiziológiai károsodást okozni és olyan faktorokként is megjelenhetnek, amik kiválthatják a sejtszintű öregedést („cellular senescence”; Chiche et al., 2020). A diploid sejtekben a replikatív élettartamuk végén jelenik meg ez a stabil sejtciklus gátolt állapot, mely az öregedés egyik fő ismérve (Chiche et al., 2020). Az öregedő sejtek osztódási képességét (részleges) újraprogramozás segítségével helyre lehet állítani, azonban a károsodott sejteknél nagyobb a tumorok kialakulásának kockázata, mely többek között hátterében állhat az öreg sejtek inkább szervezetben történő felhalmozódásának (Chiche et al., 2020). Ahogyan a sejtszintű öregedés példája és az eddigi tanulmányok nagy része megerősíti, az öregedés folyamatával párhuzamosan a fiziológiás regeneráló folyamatok csökkenése is általánosan jelentkezik (Yun, 2015). Éppen ezért, egyes elméletek szerint az öregedés a regenerációs képesség csökkenésének és a megfelelő funkciójú sejtek és szövetek fenntartási elégtelenségének következménye (Sharpless & DePinho, 2004). Ezt az elgondolást fordított szcenárióval igazolja azoknak az élőlényeknek a létezése, melyek regenerációs képességei igen rendkívüliek és mérhető funkcionális hanyatlással nem rendelkeznek, amik az elhanyagolható öregedés jeleinek tekinthetők (Finch, 1994). Sőt, az erős javító és regenerációs képességű fajoknál a negatív öregedés (vagyis az életkorral csökkenő mortalitás és növekvő fekunditás jelensége) is előfordulhat, általában olyan jellemzőkkel társulva mint a folyamatos oogenézis, nagy mennyiségű őssejtek, a szóma és csíravonal sejtek elkülönülésének hiánya vagy a klonális szaporodásra való képesség (Vaupel et al., 2004).

1.1.6. Forrásallokáció szerepe az öregedés során

Ahhoz, hogy az öregedés radikálisan eltérő formáit, mint pl. a negatív öregedést megértsük, meg kell vizsgálnunk az öregedés alapvető okait (Baudisch & Vaupel, 2012). Ehhez jó megközelítés az allokációs elmélet, mely azt igyekszik tisztázni, hogy a forrás limitáltság hogyan befolyásolja az életmenet jellegeket (Baudisch & Vaupel, 2012). Eszerint, az evolúciós fitnesz kialakítása az egymással forrásokért versengő, túlélést és/vagy szaporodást segítő folyamatokba való optimális forrásallokáción alapszik (Davison et al., 2014). Ennélfogva ebben a rendszerben minden döntés (vagyis kogníció vagy akarat nélküli stratégiai választás) a csereviszonyok révén túlélést, szaporodást és növekedést érintő következményekkel jár (Davison et al., 2014). Habár az öregedés elméletek elismerik az allokációs csereviszonyok meglétét, elhanyagolják azok alapvető fontosságát és a nemi érettséget követő, korral csökkenő szelekciós hatásra (vagyis szelekciós árnyékra) fókuszálnak (Baudisch & Vaupel, 2012). Ez is közrejátszhat abban, hogy keveset tudunk az öregedő és nem öregedő mintázatokat kiváltó tényezőkről (Baudisch & Vaupel, 2012). Ilyen tényező lehet például a túléléshez szükséges minimum metabolikus befektetés vagy a források elérhetősége is (Davison et al., 2014). A források elérhetősége, limitáltsága összefüggésbe hozható többféle túlélést és szaporodást érintő változással. Tipikus példa az étrendi és kalória korlátozás élethossz növekedéssel társuló hatása, melyet számos egereken, *Drosophila*-n, *Caenorhabditis elegans*-on végzett kutatás támaszt alá (összefoglalva: (Kennedy et al., 2007)). Az elégtelen táplálék azonban jelzésként szolgálhat egy élethossz növekedéssel ellentétes folyamatra is, a végső befektetés („terminal investment") során ugyanis a túlélés lecsökken, míg az energiabefektetés a szaporodás intenzív növelésére irányul (Clutton-Brock, 1984; Fischer et al., 2009). Az állatvilágban azonban nagy változatosság figyelhető meg az önfenntartó folyamatokba való befektetés következményeit illetően. A javító és önfenntartó mechanismusokba való fokozott befektetés egyes fajokban csökkentik a fertilitást, másoknál csak kis hatással van rá, míg megint másoknál a jelenlegi fekunditás csökkenésével és a jövőbeli fekunditás növelésével jár együtt (Baudisch, 2012). E változatosság tanulmányozása az öregedő és nem öregedő mintázatok kialakulásához és az öregedés evolúciójának megértéséhez is közelebb vihet, ezért nagyobb kutatási hangsúlyt érdemelnének (Baudisch & Vaupel, 2012). Ennek a változatosságnak a megragadására potenciálisan alkalmas a *Hydra* nemzetség, ugyanis a genus tagjait általában elhanyagolható öregedéssel rendelkezőknek tartják (Dańko et al., 2015), azonban egyik fajuk (*Hydra oligactis*) ivaros szaporodás után öregedésre jellemző intenzív degenerációt és csökkent túlélést mutat (Yoshida et al., 2006). Ezért is fontos, hogy az öregedést mutató fajon végzett 3. tanulmánnyal bővítsük a forrásallokációt, szaporodást és túlélést egyszerre érintő tudásunkat.

1.1.7. Édesvízi hidrák, mint modell szervezetek

Habár az elmúlt bő egy évtizedben a hidrák a halhatatlan jelzőt kiérdemelve konstans fertilitásuk és mortalitásuk révén keltettek tudományos érdeklődést (Martínez, 1998; Schaible et al., 2015), kutatásuk Abraham Trembley 1744-es regenerációs vizsgálatával kezdődött. Trembley vizsgálati kérdése az volt, hogy eldöntse a zöld hidrákról (*Hydra viridissima*), hogy növényeknek vagy állatoknak tekinthetőek-e. Úgy vélte, hogyha félbevágást követően mindkét fél regenerálódik akkor növényekről, ha a regeneráció elmarad akkor állatokról van szó. Kísérlete mindkét testrész regenerációjával zárult, közben azonban másik egyeden sikerült megfigyelnie zsákmányszerzésüket, így - helyesen - állatoknak tekintette a hidrákat (Lenhoff & Lenhoff, 1988). Az azóta eltelt több mint 270 év alatt tudásunk a hidrákról rengeteg információval bővült. Ma már ismeretes, hogy a *Hydra* genushoz néhány tucat, apró méretű, ragadozó életmódot folytató faj tartozik (Kawaida et al., 2010). Ezekből 5 faj fordul elő Európában (Schuchert, 2010) és leginkább két faj, a közönséges hidra (*Hydra vulgaris*) és a nyeles hidra (*Hydra oligactis*) áll intenzívebb kutatás alatt. A hidrák közös jellemzője, hogy testük nagy arányban tartalmaz őssejteket: endotél-, epitél- és sejtközötti őssejteket, utóbbiak multipotensek mivel az ideg-, csalán-, mirigy- és csíravonal sejtek képzéséért is felelnek (Nishimiya-Fujisawa & Kobayashi, 2012). Az őssejtek osztódása révén a hidrák törzsüktől karjaik és talpuk felé tolódva folyamatosan lecserélik sejtjeiket, ezzel biztosítva szervezetük megújulását, intenzív fiziológiás regenerációját (Campbell, 1967a, 1967b). Ivartalan szaporodásra való képességük tovább erősíti a nagy szöveti plaszticitás meglétét, mivel emiatt is szükség van a folyamatos növekedésre és a sejtek osztódására („turnover"-ére) (Holstein et al., 2003). Az intenzív őssejtosztódás, a csíra- és szomatikus sejtvonal teljes szeparáltságának hiánya és a klonális szaporodás képessége, mind elősegítheti a *Hydra vulgaris*-nál és *Hydra magnipapillata*-nál tapasztalható elhanyagolható öregedést (Martínez & Bridge, 2012; Orive, 1995), melyhez továbbá hozzájárulhat, hogy őssejt aktivitásuknak köszönhető regenerációs képességük is (Chera et al., 2009; Govindasamy et al., 2014). A fiziológiás regenerációjuk mellett a sérülés indukálta regenerációs képességük is igen figyelemre méltó, ugyanis a hidrák akár eredeti méretükhöz képest mindössze 1%-os szövetdarabból is képesek regenerálni a teljes testüket (Shimizu et al., 1993). Az eddigi hidrákon végzett regeneráció kutatások főleg a folyamat fiziológiai, fejlődési és molekuláris aspektusait vizsgálják (Bode, 2003; Kemmner & Chica Schaller, 1984; Tardent, 1974). A regenerációval mint életmenet jelleggel való összefüggéseket viszont sokkal kevésbé értjük, ezért munkámmal erre a területre fókuszáltam.

A regeneráció hidrák esetében is egy plasztikus jelleg, amire példaként szolgál a nyeles hidra (*Hydra oligactis*), mely ivaros egyedeinél késleltetett vagy gátolt regenerációt írtak le (Galliot et al., 2018; Tomczyk et al., 2017). A regenerációs képességük elvesztésének hátterében vélhetően a szaporodási módjuk áll: ősszel a hőmérséklet csökkenés hatására ivartalan szaporodásról ivarosra váltanak, hogy más *Hydra* fajokhoz képest nagy számban hozzanak létre a zord időjárási körülményeknek ellenálló kitartó petéket (Reisa, 1973; Schuchert, 2010). A többi fajtól eltérően a nyeles hidráknál az ivaros szaporodás alatt és azt követően jelentős változások tapasztalhatók, ugyanis az ivarsejtek számának növekedésével a sejtközötti (intersziticiális) őssejtjeik száma erősen csökken, majd ivaros szaporodás után testük degenerálódik és elpusztulnak (Tardent, 1974; Yoshida et al., 2006). A drasztikus változások miatt az öregedést az ivaros szaporodás következményének tartják, melyet őssejtjeik ivarsejtekké való intenzív differenciálódása során való kimerülése és így a szomatikus funkciók ellátásának gátoltsága idézhet elő (Bosch, 2009; Brien, 1966; Tardent, 1974). Meg kell azonban jegyezni, hogy ezek a megfigyelések eddig csak néhány, laboratóriumi vonal alapján születtek, befolyásoló tényezőit (pl. táplálék vagy életkor) nem vizsgálva. Továbbá, a természetes populációkban megjelenő szaporodási stratégiákkal és azokban a sejtszintű forrásokért való versengés mediátor szerepével sem vagyunk tisztában, mely kérdéseket a 1., 2. és 3. tanulmányokban vizsgáltunk meg. Tekintve, hogy öregedő és nem öregedő folyamatok vizsgálata lehetséges hidráknál, akár közelrokon fajok összehasonlító viszgálatával, de nyeles hidráknál fajon belül is lehetséges. Így a hidra fajok ideális modellszervezetként szolgálnak a regeneráció, öregedés és ezek életmenet jellegeket érintő következményeinek tanulmányozására, melyek disszertációs munkám alapvető kérdésköreit is alkotják. Disszertációm mindhárom vizsgálatának modell szervezete a nyeles hidra volt, mely a fenti adottságai miatt kiváló alany a kérdéseim megválaszolásához.

1.2. Célkitűzések

1.2.1. Őssejt készlet és a szaporodási módok szerepe (1. tanulmány)

Multifuncionalitásuknak köszönhetően az őssejtek akár eltérő életmenet komponenshez tartozó jellegek kialakításában egyszerre játszhatnak meghatározó szerepet. A szaporodás az őssejt forrásokat intenzíven felhasználhatja, ezzel csereviszonyi kapcsolatokat eredményezve más, olyan önfenntartásért felelős jellegekkel, mint amilyen a regeneráció is, amely szintén az őssejtekre támaszkodik. Az ivaros és ivartalan szaporodási módok közötti különbség azonban az őssejt igényük tekintetében is megjelenhet, és így eltérő hatással lehet a két szaporodási mód a többi jellegre. **A disszertációm ezen részében az ivaros és ivartalan szaporodási módok, az őssejt források elérhetősége, és az őssejtektől függő regeneráció valamint csalánsejt szám csereviszonyi kapcsolatok viszgálatát tűztem ki célul.** Ennek keretében végzett kutatásunk fő kérdései az alábbiak voltak:

(1) A fejregenerációs képesség eltér-e a különböző szaporodási módokban?

(2) A fejregenerációs képesség változása összefügg-e az őssejtek számának változásával?

(3) Van-e különbség az őssejtek és csalánsejtek számában az eltérő szaporodási módokban (nem szaporodó, ivartalan, ivaros)?

(4) Az ivarsejtek száma valamint az őssejtek és csalánsejtek száma között tapasztalható-e korreláció természetes populációból származó egyedekben?

Nyeles hidrákat természetes élőhelyükről gyűjtöttünk annak érdekében, hogy egy ilyen populációról kaphassunk képet. A nyeles hidrákról néhány korábbi tanulmány alapján ismeretes, hogy ősszel a populáció egy része képes ivartalan szaporodásról ivaros szaporodásra váltani, és a szaporodási módban való váltást követően az egyedek degenerálódnak és elpusztulnak. Az ivaros utódok téli túlélésefeltételezhetően nagyobb, mivel a kitartó peték a faggyal szemben ellenállóak.

Predikciónk szerint az ivaros egyedek (i) többet fektetnek a szaporodásba (ii) a többlet szaporodási befektetés csökkent őssejt készletetet eredményez, és (iii) az őssejtek mediátor szerepének köszönhetően az önfenntartási jellegekre, így a regenerációra és csalánsejt képzésre fordítható források elérhetősége is csökken a ivartalanul szaporodó egyedekhez képest. Továbbá, (iv) a szaporodás és az önfenntartás csereviszonya esetén az ivarsejtek és az őssejtek száma között is negatív korreláció prediktálható.

1.2.2. Korfüggő plaszticitás szaporodásban és önfenntartásban (2. tanulmány)

Az alapvető életmenet jellegek gyakran kor- és életszakasz-függő plaszticitást mutatnak. Az ivartalan szaporodási módnak köszönhetően, a klonális vagy parciálisan klonális álloknál azonban a korfüggő életmenet döntések és prioritások igen összetettek lehetnek, a konvencionális modellektől eltérő regenerációs és szaporodási mintázatokat mutatva. A parciálisan klonális állatok esetében az ivartalanról ivaros szaporodási módra való váltás indukálása és időzítése is egy további fontos életmenet döntés. Míg az ivaros és ivartalan szaporodási módok közötti különbségek viszonylag intenzíven kutatottak, arról sokkal kevesebbet tudunk, hogy a korral változó szaporodási módokhoz köthető forrásallokációs mintázatok milyen regenerációs és önfenntartásbeli változásokkal társulnak klonális vagy parciálisan klonális állatok esetében. **Éppen ezért, nyeles hidrákon végzett vizsgálatunkban, a szaporodáshoz (gametogenézis és ivartalan szaporodás) és önfenntartáshoz (fejregenerációs képesség és túlélés) kapcsolódó életmenet jellegek korfüggő plaszticitását vizsgáltuk, az ivaros szapordás hőmérséklet csökkenés hatására történő indukálása révén .** Kísérletünk célja az alábbi fő kérdések megválaszolása volt:

(1) Az ivaros és ivartalan szaporodási módokkal párhuzamosan változik-e a regenerációba és túlélésbe való befektetés fiatal (1 hetes) és idősebb (4 hetes) egyedekben?

(2) Hogyan változik a két korcsoport fejregenerácós képessége és őssejtjeinek a száma az ivaros szaporodást indukáló hőmérséklet csökkenést követően?

(3) A fiatalabb és idősebb egyedek ivaros szaporodásának időzítésében (kezdetében) és intenzitásában (ivarszerveinek számában) is tapasztalható eltérés?

(4) A regenerációban és szaporodásban tapasztalt korfüggő, fenotipikus változásokat alátámasztják-e a sejtípusokban (őssejtek, ivarsejtek) mérhető mennyiségi értékek?

Ha az általános életemenet elmélet szerint a fekunditás a korral növekszik és a szomatikus sejtek fenntartása viszont csökken, akkor az idősebb állatokban az ivaros szaporodásért felelős szervek és ivarsejtek száma várhatóan nagyobb, míg regenerációs képességük, túlélésük és intersticiális őssejtjeik száma kisebb, mint a fiatalabb egyedeknél. Ezzel ellentétesen azonban az is lehetséges, hogy az idősebb egyedek hosszabb élettartamuk alatt a források felhalmozása révén képesek mindkét életmenet jellegbe nagyobb mértékben befektetni anélkül, hogy csereviszony lenne közöttük. Az ivartalan szaporodás priorizálása néhány korábbi megfigyelés alapján inkább a fiatalabb korcsoportban várható, ám az idősebb egyedek jobb kondíciójának köszönhetően akár ellentétes értékek is lehetségesek.

1.2.3. Forrásallokáció ivaros szaporodás és túlélés között az öregedés hátterében (3. tanulmány)

Az öregedés egyik jó indikátora, hogy a túlélést nagyban befolyásoló sejti és szöveti megújulás képessége, azaz a fiziológiás regeneráció csökken. Ez gyakran tapasztalható az ivaros szaporodást követő életszakaszban, melynek egyik oka a forrásallokációban keresendő. A forrás limitáltságon alapuló csereviszonyi kapcsolatokon keresztül az életmenet döntések mindkét fő életmenet komponensre, a szaporodásra és az önfenntartásra is kihatnak. Az életmenet döntések kimenetelének a források elérhetőségének függvényében azonban változó következménye lehet. **Disszertációs munkám ezen részében, az intenzív posztreproduktív degenerációt és öregedést mutató nyeles hidránál vizsgáltuk a táplálék elérhetőség hatását az ivaros szaporodásra és túlélésre.** Vizsgálatunk kapcsán az alábbi központi kérdésekre kerestük a választ:

(1) Milyen hatással van a táplálék elérhetősége (4 táplálékmennyiség szinten) az ivaros szaporodás megjelenésére, az ivarszervek számára és a túlélési rátára?

(2) Összefügg-e az ivaros szaporodás mértéke a későbbi túlélési rátával?

(3) Tapasztalható-e eltérés a különböző, természetből származó nyeles hidra vonalak között az ivaros szaporodásra való hajlam, az ivaros szaporodás mértéke és az azt követő túlélési ráta tekintetében?

Ha két életfunkció (szaporodás és önfenntartás) egyforma mértékben fontos, akkor mértékükben hasonló csökkenés várható alacsony táplálék szintek esetén. Ha azonban a funkciókhoz tartozó jellegek nem egyenértékűek, akkor egyik vagy másik prioritása feltételezhető. Ennek a jelenségnek az extrém kimenetelei jelenhetnek meg az étrendi korlátozás („dietary restriction", csökkent fekunditás és megnövekedett túlélés) vagy a végső befektetés („terminal investment", intenzív fekunditás és csökkent túlélés) esetén is.

1.3. Anyag és módszerek

1.3.1. Őssejt készlet és szaporodási módok szerepe (1. tanulmány)

A nyeles hidra egyedeket természetes élőhelyükről, egy Tiszadorogma melletti Tisza holtágból gyűjtöttük az őszi-téli szaporodási időszak alatt, négy alkalommal. A víz alatti vegetációról begyűjtött egyedeket Eppendorf csőbe helyeztük és hűtőládában még aznap a laborba szállítottuk. A következő napon az egyedek felénél, akiket randomizálva választottam ki, megkezdtem a fejregenerációs mérést. Ennek első lépéseként eltávolítottam a feji részt, majd négy nap múlva bináris rendszer szerint, az új tapogatók jelenléte vagy hiánya alapján értékeltem a regenerációt (van tapogató = regeneráció). Az állatokat ez alatt klímakamrában, a természetes élőhelyükön mért hőmérsékletnek megfelelően tartottam. Az állatok egy másik részét sejtösszetétel vizsgálatnak vetettük alá. A standard eljárás szerinti a macerálást a gyűjtés utáni napon elvégeztük, később pedig megszámoltuk az alábbi sejttípusokat: epitél sejtek, intersticiális őssejtek, nematoblaszt (csalánsejt) sejtcsoportok és reproduktív sejtek (sperma prekurzor sejtcsoportok a hímeknél és dajka sejtek a nőstényeknél). A statisztikai elemzéshez Általánosított Lineáris Kevert Modelleket (Generalized Linear Mixed-effects Models, GLMM) használtam, az adatoknak megfelelő hiba eloszlással (Bayesian környezetben ahol szükséges volt).

1.3.2. Korfüggő plaszticitás szaporodásban és önfenntartásban (2. tanulmány)

A kísérletben használt hím és nőstény nyeles hidra vonalak egy Tiszadorogma melletti holtágból származtak, amiket törzstenyészetben tartottunk a kísérletet megelőző egy évben.

Az állatokat standard hidra médiumban, állandó körülmények között, 18 Celsius fokon, klímakamrában tartottuk. A kísérletben frissen levált bimbókat használtunk. Kísérleti kezelésként hőmérséklet csökkentést alkalmaztunk (7 Celsius fokra) 3 vagy 24 nap elteltével a kísérlet megkezdése után. Az állatokat heti több alkalommal megvizsgáltuk, hogy rögzítsük ivartalan szaporodási rátájukat és ivarszerveik számát. A regeneráció mérését két hónapon át hetente elvégeztem az állatok egy részén, a feji rész eltávolítását követően egy héttel, az új tapogatók megjelenések bináris kódolása szerint (igen/nem). A sejtösszetétel méréseket szintén két hónapon át körülbelül hetente elvégeztük, az epitél sejtek, az intersticiális őssejtek, a sperma prekurzor sejtcsoportok és a dajka sejtek számának feljegyzése révén. A kísérleti állatokat 5 hónapon át tartottuk, feljegyezve a haláleseteket, binárisan kódolva a túlélést. Az adatok elemzéséhez Általánosított Lineáris Kevert Modelleket használtam megfelelő hiba eloszlással, melyeket 'Likelihood ratio test' követett.

1.3.3. Forrásallokáció vizsgálata ivaros szaporodás és túlélés között az öregedés hátterében (3. tanulmány)

A kísérletben használt 10 nyeles hidra vonal létrehozásához szükséges 10 hidra egyed a Tiszadorogmához közeli holtágból származott. A 10 állat gyűjtését követően azokat standard laboratóriumi körülmények között tartottuk 20 Celsius fokon és két hónap alatt az egyedeket ivartalanul felszaporítottuk. A kísérlet ideje alatt az állatokat heti háromszor etettük, 4 kezelési csoport szerint, melyek táplálékmennyiségben tértek el egymástól (3, 6, 10 vagy 20 µl Artemia sórákot adtunk etetési alkalmanként). A kísérlet kezdetétől számított második héten a hőmérsékletet 7 Celsius fokra csökkentettük, annak érdekében, hogy indukáljuk az ivaros szaporodást. Az ivaros szaporodás mértékének méréséhez rögzítettük az ivarszervek számát (leesett petéket a nőstényeknél, testiseket a hímeknél). Az állatok halálát is rögzítettük amennyiben azok szétestek vagy eltűntek. Különösen ügyeltünk arra, hogy az igen kisméretűre összement állatokat is megtartsuk. Az állatokat 150 napig tartottuk, amikor is a legtöbb állat már halott volt vagy regenerálódott az ivaros szaporodás után és legalább egy ivartalan utódott hozott. A statisztikai elemzéshez Általánosított Lineáris Modelleket (Generalized Linear Models, GLM) vagy Általánosított Lineáris Kevert Modelleket (GLMM) és 'Likelihood ratio test'-et alkalmaztunk Poisson vagy binomiális hiba eloszlással.

1.4. Eredmények

1.4.1. Őssejt készlet és szaporodási módok szerepe (1. tanulmány)

- Az ivaros egyedek regenerációs képessége, valamint csalánsejt és intersticiális őssejt készlete is csökkent az ivartalanul szaporodó egyedekhez képest. Az ivartalan és nem szaporodó egyedek között azonban nem volt különbség.
- Az ivaros egyedekben mért ivarsejt szám negatívan korrelált az intersticiális őssejtek és csalánsejtek számával.
- Regenerációs képességre és sejtösszetételre vonatkozó eredményeink igazolják az ivaros és ivartalan szaporodási módok eltérő fiziológiai költségét.

- Eredményeink megerősítik a regeneráció életmenet jellegként való értelmezését, továbbá azt az elméletet, miszerint az őssejtekért folyó kompetíció mediátor szereppel rendelkezik az életmenet döntésekben.
- Természetes populációból gyűjtött állatoknál is igazoltuk az eddig csak néhány, régóta laborban tartott vonalaknál tapasztalt, ivaros szaporodást követő csökkenő csalán- és őssejt mintázatokat.

1.4.2. Korfüggő plaszticitás szaporodásban és önfenntartásban (2. tanulmány)

- Igazoltuk a korfüggő plaszticitás meglétét az összes, nyeles hidrákon mért változóban (fejregenerációs ráta, ivartalan szaporodási ráta, ivarszervek száma, ivaros szaporodás kezdete, túlélés, ivarsejtek száma, őssejtek száma).
- A fiatalabb egyedek magasabb ivartalan szaporodással, míg az idősebb egyedek intenzívebb gametogenezissel és ivarsejt képzssel rendelkeztek.
- A szaporodásban mutatott eltérésekkel párhuzamosan, az önfenntartási jellegekben is eltérés mutatkozott kor függvényében: az idősebb, inkább ivarosan szaporodó egyedek alacsonyabb fejregenerációs képességgel, túléléssel és őssejt számmal rendelkeztek.
- A fejregenerációs ráta és az őssejtek számának változása plasztikus korfüggő hatás mellett ontogenetikus hatásra is utal, ugyanis a fiatalabb egyedek az ivaros szaprodást a fiatalabb és az idősebb egyedek közötti korkülönbséghez hasonló mértékű késéssel kezdték meg.

1.4.3. Forrásallokáció vizsgálata ivaros szaporodás és túlélés között az öregedés hátterében (3. tanulmány)

- Az ivaros szaporodást csökkent túlélés követte, azonban a korábbi kísérletekkel ellentétben, az állatok egy jelentős része regenerálódott az ivaros szaporodás utáni degenerációt követően és ivartalanul is tovább szaporodott. Ez az állatvilágban kivételes életmenetnek számít és kivételes megnyilvánulása a szaporodás és túlélés közötti csereviszonynak is.
- A táplálékmennyiség nem volt hatással az ivaros szaporodás megkezdésére, az ivarszervek számára viszont igen, ugyanis a magas táplálékszint nagyszámú ivarszerv képzését eredményezte.
- A túlélési rátát nem befolyásolta az elérhető táplálék mennyisége, ezzel azt sugallva, hogy a túlélés egy konzervált jelleg, fenntartása alacsony táplálékszint esetén is fontos.
- Az ivarszervek száma és a túlélés között nem volt egyedszintű összefüggés.
- A 10 nyeles hidra vonal között eltérés mutatkozott azok ivaros szaporodásra való hajlamában, ivarszerveinek számában és túlélésében is. Az ivaros szaporodást csak kis arányban vagy egyáltalán nem megkezdő vonalat is találtunk.

1.5. Diszkusszió

Munkám során a regenerációt, mint széles körben megjelenő életmenet jelleget vizsgáltam, szaporodáshoz és önfenntartáshoz kapcsolódó jellegekkel kapcsolatos kutatásokat elvégezve. A regenerációt és a hozzá kapcsolódó jellegeket három fő tényezővel összefüggésben vizsgáltam édesvízi hidráknál: (i) őssejt források, (ii) életkor és (iii) táplálék elérhetőség. Mind a három tényező függvényében szoros összefüggéseket fedeztünk fel a két fő életmenet komponsekhez, a szaporodáshoz és a túléléshez tartozó jellegek valamint a regeneráció között. Továbbá két esetben megfigyeltünk ivaros és ivartalan szaporodási módok közötti különbségeket is. A következőkben fő vizsgálati tényezők, kontextusok szerint értelmezem a jelentősebb eredményeinket.

Az őssejt források tekintetében, természetes élőhelyükről gyűjtött nyeles hidráknál az eltérő szaporodási módú csoportokban markáns különbségek mutatkoztak (1. tanulmány). Az ivaros egyedek csökkent fejregenerációs képességekkel, őssejt számmal és a táplálkozásban szerepet játszó csalánsejt készlettel rendelkeztek a nem szaporodó egyedekhez képest, míg az ivartalan csoport nem mutatott eltérést a nem szaporodóktól, ellenben különbözött az ivarosan szaporodóktól. Az ivaros szaporodással összefüggő csökkent szomatikus funkciók (regeneráció, őssejtek, táplálkozás) evolúciós oka lehet egy olyan szelekciós hatás, mely a hosszabb távú túlélés helyett a fiatalkori vagy jelenlegi szaporodást priorizálja. Az öregedés evolúciós elméletei szerint, magas külső tényezők okozta mortalitás esetén ugyanis a túlélésre káros mutációk elterjedhetnek a populációkban (mutáció felhalmozódás elmélet: Medawar, 1952, antagonista pleiotrópia elmélet: Williams, 1957, eldobható test elmélet: Kirkwood & Rose, 1991). A potenciálisan megnövekedett mortalitás mérsékelt övi hidra populációkra is igaz, a hideg idő és a fagy beállta különösen a sekély vizekben jelent veszélyt. Ilyen esetekben a megfagyásnak is ellenálló kitartó peték létrehozása nagyobb reprodukciós értékkel bír az ivartalan utódokkal szemben, így szelekció hatására elterjedhet az ivaros szaporodásba való intenzív befektetés, akár a szomatikus funkciók fenntartásának kárára is. Az ivartalanul szaporodó egyedek stratégiája ezzel szemben azon alapulhat, hogy az alacsony szaporodási értékkel rendelkező utódaik létrehozása alacsony költséggel is jár. Ezek fényében, a szaporodás és a túlélés közötti csereviszony várhatóan az ivaros egyedeknél hangsúlyosabb és a hátterében állhat a nyeles hidráknál is tapasztalt öregedési és szomatikus funkciókat érintő mintázatok megjelenésének, mely jelenség fiziológiai alapját a limitált őssejt készletek elérhetősége jelentheti.

Nagy szöveti plaszticitású állatoknál a multipotens őssejtek számos életfunkció kialakításában részt vesznek, ezért az egyik funkcióba való intenzív befektetés az őssejt források kimerülése révén más funkciók csökkentésével járhat együtt. Ez a jelenség nem mélyrehatóan kutatott terület, azonban korábbi vizsgálatokból már tudjuk, hogy intenzív, többszöri ivaros szaporodás után az őssejtek elhasználása *Aurelia* polipok esetében is megfigyelhető (Gold & Jacobs, 2013), valamint az őssejtek csökkent elérhetősége bizonyára felelős a *Tubularia* csalánozók élethosszának meghatározásáért (Tardent, 1963). Nyeles hidrák esetében is valószínűleg az őssejtek kimerülése (másnéven gametikus krízis; (Bosch, 2009; Brien, 1966)) játszik szerepet az ivaros szaporodást követően tapasztalt csökkenő túlélésben (Yoshida et al., 2006). A nagyobb reprodukciós befektetés során ugyanis a nagy számú ivarsejtképzés az őssejtek nagyarányú elköteleződését igényli a csíravonal útvonal iránt, ami az őssejtek limitáltságából adódóan a szomatikus funkciókat ellátó sejtek és magának az őssejt készletnek a csökkenésével járhat. Ezt vizsgálatunk sejtszám mérései is alátámasztják, mivel az ivarsejtek száma és őssejtek valamint csalánsejtek között negatív korrelációt találtunk hímek esetében. Érdekes módon, nőstények esetében nem volt tapasztalható ilyen összefüggés. Ennek oka egyfelől lehet az, hogy a csíravonal és a szomatikus sejtek közötti csereviszonyi kapcsolat hímek és a nőstények esetében eltérő mechanizmusokon alapul, másfelől viszont abból is adódhatott, hogy ivarsejtek alapján a szaporodási befektetés másképp becsülhető a nemek között. Nőstények esetében ugyanis a kezdetben nagy mennyiségű dajkasejt fokozatosan egy-egy érett petesejtté egyesül (Miller et al., 2000; Zihler, 1972), ezért az ivari érés előrehaladtával a dajkasejteket létrehozó őssejtek számának csökkenésével párhuzamosan, maguknak a dajkasejteknek a száma is csökken a petesejtté alakulás során. Ezzel összhangban van, hogy vizsgálatunkban aránylag nagy volt azon nőstények száma, akik kimerítették mind az őssejt, a csalánsejt és a dajkasejt készletüket is.

Az őssejt készlettel párhuzamosan, a fejregenerációs képesség is lecsökkent az ivarosan szaporodó egyedeknél, az ivartalanoknál viszont megmaradt. Az őssejteknél és a regenerációnál tapasztalt változások összefügghetnek egymással, ugyanis az őssejtek szerepe kritikus több regenerációs típusban is, vagy azért mert osztódások révén biztosítják a regenerációhoz szükséges sejteket, vagy a sérülés helyéhez vándorolva részt vesznek az új testrész kialakításában (Alvarado, 2000; Bely & Nyberg, 2010; Sugimoto et al., 2011). Azonban, ha bármilyen fiziológiai folyamat csökkenti az őssejtek elérhetőségét, akkor várhatóan a regeneráció is korlátozottá válhat (Henry & Hart, 2005; Kramarsky-Winter & Loya, 2000). Ahogy korábbi kutatások is alátámasztják, a regeneráció része az allokációs stratégiáknak és csereviszonyi kapcsolatoknak (Gross, 1925; Rinkevich & Loyla, 1989; Zattara & Bely, 2013), valamint az őssejt források elérhetőségének a szerepe is lehetséges magyarázatként merült fel például hidrák többszöri amputációs eseményt követő mérsékelt regenerációjánál (Martinez, 1996; Tardent, 1963). Vizsgálatunk ezek megerősítése mellett rávilágít arra is, hogy a szaporodási módok eltérően hathatnak a regenerációs képességre, mely különbségek oka valószínűleg az eltérő őssejt költségükben rejlik.

Mivel a regenerációs képesség plasztikus jelleg, az életkor is lényeges befolyásoló tényezője lehet. Az életkor előrehaladtával változhatnak a priorizált életmenet jellegek, ami parciálisan klonális állatok esetében igen összetett jelenség lehet, mivel életmenetük során különböző szaporodási módok is előfordulhatnak (ivaros és ivartalan). Ezt a kérdéskört vettük górcső alá egy másik, kísérletes vizsgálatunkban (2. tanulmány). Ennek eredménye alapján elmondható, hogy a nyeles hidrák életmenet jellegei az életkor által jelentősen befolyásoltak. Az ivaros szaporodás kísérletes indukálása által felfedeztük, hogy a korfüggő szaporodási módokhoz kapcsolódó stratégiák különböző mértékű önfenntartással társulnak. Ez a kapcsolat fontos része lehet a különböző öregedési mintázatoknak is. Habár eddigi tanulmányok alapján az a tendencia látszik kirajzolódni, hogy az ivartalan szaporodású állatok gyakran elhanyagolható vagy negatív öregedéssel rendelkeznek (Vaupel et al., 2004), míg az ivaros szaporodású állatok önfenntartása az életkor előrehaladtával hanyatlani kezd (Kirkwood & Rose, 1991), vizsgálatunk rámutat arra, hogy ezek a mintázatok változhatnak az életkor során.

Ahhoz, hogy a klonális és nem klonális élőlények olyan fő életmenet komponenseket, mint a növekedést, szaporodást, regenerációt és önfenntartást érintő allokációs mintázatait és prioritásait valóban megértsük, érdemes figyelembe vennünk egy lényegi különbséget a két csoport között. Fontos lehet ugyanis, hogy klonális és parciálisan klonális élőlényeknél a növekedés nem csak a test méretbeli fokozódását jelentheti, hanem a testből szomatikusan létrehozott ivartalan utódok képzését is (Harvell & Grosberg, 1988). Ivartalan szaporodás révén az adott környezethez alkalmazkodott genotípus az ivaros szaporodásnál kisebb költséggel (Rispe et al., 1998), gyorsan képes egy élőhelyet kolonizálni. A nem klonális élőlényeknél tapasztalhatóakkal ellentétben ezért lehetséges, hogy az ivartalan szaporodásba való befektetés jó kondíció esetén már korai életszakaszban előnyös lehet (Burke & Bonduriansky, 2018). A parciálisan klonális fajoknál a genetikai diverzitás növelése pedig csak később, az ivaros szaporodás révén válhat előnyössé (Burke & Bonduriansky, 2018). Kutatásunk megerősíti ezt a feltevést, mivel azt találtuk, hogy nyeles hidrák élete során az ivartalan szaporodás fiatalabb korban, az ivaros szaporodás pedig csak később priotitás. Ezen eredményeink összhangban vannak a korábbi vizsgálatokkal, melyek például kevesebb petéről számolnak be fiatal korban nőstény botsáskáknál (Burke & Bonduriansky, 2018), korfüggő ivarérésről néhány korall fajnál (Kai & Sakai, 2008) vagy az ivaros szaporodást megelőző ivartalan ciklusról több parciálisan ivartalan faj esetében (Olive, 2002). Habár vannak példák ezekre a korfüggő szaporodási mintázatokra, a szaporodási módok közötti váltás kísérletes indukálása sokkal kevésbé vizsgált, holott a váltás befolyásoló tényezőinek, előnyeinek és költségeinek megbecsüléséhez elengedhetetlen.

Vizsgálatunk során a szaporodási módok eltérő önfenntartási költségekkel társultak: a fiatalabb egyedek, akik magasabb ivartalan szaporodási rátával és késleltetett, kevésbé intenzív gametogenezissel rendelkeztek, magasabb regenerációs és túlélési értékeket mutattak, míg ezek ellenkezője volt igaz az idősebb hidra polipokra. Az ivartalan szaporodással társuló magasabb regenerációs képesség és túlélés más parciálisan ivartalan fajnál is megjelenik (Mouton et al., 2018; Zattara & Bely, 2016), valamint az ivaros szaporodás ezekre való negatív hatása is ismert (Harvell & Grosberg, 1988; Henry & Hart, 2005). A korral bekövetkező változások tekintetében elmondható, hogy az ivartalan szaporodás képességével általában nem csökkenő regenerációs képességek és túlélés társul (Tanner, 2001; Yun, 2015), azonban egyes esetekben ezek csökkenése is előfordulhat (Meesters & Bak, 1995; Orive, 1995; Sköld et al., 2011). Ezen jelenségre is magyarázatul szolgálhat, hogy az önfenntartás korral történő változásai más életmenet jellegektől is függnek (Orive, 1995), mint például a vizsgálatunkban is szereplő szaporodási módoktól. A korábbi tanulmányok azonban a két szaporodási mód és az önfenntartási jellegek kapcsolatát általában nem egyszerre, egy fajon belül vizsgálják, és nem foglalkoznak az életkor hatására bekövetkező változásokkal, mely fontosságára eredményeink is rámutatnak.

Proximális szinten nézve, sejtszintű méréseink lehetséges magyarázatot adhatnak a korral növekvő ivaros szaporodásra való hajlandóságra és annak nagyobb költségére is. Az ivarsejtek előállítása hidráknál a csíravonal őssejtek révén történik, melyek a multipotens őssejtekből származtathatóak (Nishimiya-Fujisawa & Kobayashi, 2012). A multipotens őssejtek egyszerre a szomatikus sejtek (csalán-, mirigy- és idegsejtek) és csíravonal őssejtek képzéséért is felelősek. Ebből adódóan, az ivarérés következtében a multipotens őssejtek differenciálódása a szomatikus sejtek helyett az ivarsejt képzési útvonal felé tolódhat. Ezen csökkent elérhetősége az őssejteknek a szomatikus funkciók felé az ivaros szaporodás egyik fő költségét is jelentheti, mivel szomatikus sejtek felelősek a regenerációért, ivartalan szaporodásért és általánosságban a test fenntartásáért is (Nishimiya-Fujisawa & Kobayashi, 2012). Nőstény nyeles hidrák esetében korábban dokumentáltak már korral növekvő csíravonal őssejt számot (Littlefield, 1991), mely egy bizonyos készültséget jelez az ivaros szaporodásra. Ennek fényében lehetséges, hogy kísérletünkben a fiatalabb állatok a megfelelő mennyiségű csíravonal őssejt hiányában nem voltak képesek nagy számú ivarsejt produkcióra sem. Az ivarsejtek és őssejtek időbeli dinamikája, a regenerációs mérésékkel együtt eredményeink további lehetséges magyarázataként az ontogenetikus hatás szerepét is felvetik. A fiatalabb, ‘1 hetes’ csoportban ugyanis ezen változók értékeiben egy 3 hét csúszás figyelhető meg a ‘4 hetes’ csoporthoz képest. Ugyanakkor, ha az állatok abszolút korára (szülő állatról való leválás időpontjától számított korára) is kontrollálunk, az ivaros szaporodás indukálása szerinti életkort érintő kezelés hatása így is megmarad. Így összességében megállapítható, hogy az ontogenetikus hatás és korfüggő plaszticitás együttesen játszhatott szerepet a nyeles hidrák életmenet stratégiáiban tapasztalt eltéréseiben.

Az életkor előrehaladtával, az öregedés során csökkenő tendencia tapasztalható a fiziológiás regenerációban (Yun, 2015), mely a túlélés egyik kulcsa. Az életkor hatását az öregedés elméletek is figyelembe veszik, a források elérhetősége ilyen szempontból azonban kisebb tudományos figyelmet kap (Baudisch & Vaupel, 2012). Az allokációs elmélet szerint, a táplálék jelenti az életmenet jellegek kifejeződésének és működésének egyik alapvető forrását, ami a hidrák érdekes életmenet stratégiáival is összefüggésben állhat. Ezt vizsgáltuk meg harmadik kísérletünkben (3. tanulmány), melyben méréseink a posztreproduktív öregedéssel rendelkező nyeles hidra faj ivaros szaporodására és az azt követő túlélésére irányultak 4 táplálékmennyiség szint bevonásával, 10 ivartalanul felszaporított hidra vonal esetében.

Kísérletünkben az elérhető táplálék mennyiségének növekedésével az ivarszervek száma is növekedett, azonban a gonadogenezis megkezdésének időpontja a táplálékelérhetőségtől függetlennek bizonyult, ahogyan az ivaros szaporodást követő túlélés is. Ezek alapján megállapítható, hogy - az alkalmazott táplálékszintek esetében - a nyeles hidrák képesek megőrizni túlélési képességüket ivaros szaporodás esetén is. Ezen eredményeink több szempontból is meglepőek. Egyfelől, nyeles hidráknál az ivaros szaporodás egyfajta vészhelyzeti stratégia, és a kitartó peték létrehozását a fagy és/vagy az éhezés miatti magas mortalitás teszi szükségessé. Kísérletünk megfigyelései alapján ez a hipotézis nem magyarázhatja teljesen a jelenséget, mivel a táplálék mennyisége nem volt hatással az ivaros szaporodás megkezdésének valószínűségére (Kaliszewicz és Lipinska (2012) eredményeihez hasonlóan). Az sem támogatja a fenti hipotézist, hogy hideg hőmérsékleten az ivartalan szaporodás nem volt folyamatosan gátolt, és az ivaros szaporodás megkezdése előtt is képesek voltak az ivartalan utódok, vagyis bimbók létrehozására. Ez azt sugallja, hogy a hőmérséklet csökkenés inkább jelként szolgál az ökológiailag optimális ivaros szaporodás megkezdésére, mintsem fiziológiai kényszer lenne (Reisa, 1973).

Meglepő továbbá, hogy a vizsgálatban szereplő kísérleti állatoknál tapasztalható volt a korábban leírtakhoz (Yoshida et al., 2006) hasonló ivaros szaporodást követő degeneráció, test- és tapogatókar méretbeli csökkenés és elhalálozás, azonban az állatok jelentős része ezt követően túlélt, regenerálódott és újra ivartalanul szaporodott. Ez a mintázat nem magyarázható sem a "terminal investment", sem a "dietary restriction" jelenségeivel, valamit olyan modellel sem, melyben a szaporodás és a túlélés egyaránt függ a táplálék elérhetőségétől. Ahogy az előző kísérleteknél (1. és 2. tanulmány) említésre került, az intenzív posztreproduktív degeneráció fő oka valószínűleg az őssejtek kimerülése (Bosch, 2008; Brien, 1953; Reisa, 1973; Tardent, 1974; Yoshida et al., 2006). A "gametikus krízis" elmélete alapján a magas posztreproduktív túlélési rátára kézenfekvő magyarázat, hogy ezeknél a vonalaknál valószínűleg az őssejtek kisebb arányban köteleződtek el a csíravonal útvonal felé és az őssejtek egy kellően nagy populációja megmaradt ahhoz, hogy a teljes test regenerációját lehetővé tegye. Tekintve a hidrák teljes test regenerációjához szükséges igen alacsony szövet méretét (Shimizu et al., 1993), ez egy potenciális magyarázatnak tekinthető.

Harmadsorban, eredményeink nem támogatják azt a hipotézist, miszerint a posztreproduktív degeneráció a táplálék források szomatikus funkciók ellátására történő csökkent allokációjának a következménye. A túlélési rátára ugyanis nem volt hatással az elérhető táplálék mennyisége, valamint nem találtunk egyedi szintű összefüggést az ivarszervek száma és a túlélés között, ami pedig a kulcs eleme a csereviszonyi kapcsolatra épülő hipotézisnek. Lehetséges azonban, hogy a mintázott vonalak száma nem volt elegendő egy ilyen kapcsolat detektálásához.

Mindazonáltal, a vizsgálatba bevont tíz, azonos populációból származó nyeles hidra vonal rávilágít a fajon belüli eltérő életmenet stratégiák lehetséges jelenlétére. Még ezen a viszonylag korlátozott vonal számon belül is akadt olyan klónokból álló vonal, amely nagy arányban szaporodott ivarosan és előfordult olyan is, ami végig csak ivartalanul (eredmények nem kerültek bemutatásra). Továbbá, egyes vonalak túlélési valószínűsége igen magas volt, míg másoké kevesebb mint 25%. Ezek az eltérések részét képezhetik eltérő életmenet stratégiáknak vagy lehetséges, hogy az időszakosan változó, életmenet jellegekre ható szelekció is eredményezheti (Mojica et al., 2012). A sok vonalnál tapasztalt, ivaros szaporodást követő túlélési arány inkább az iteropár szervezetekre jellemző, melyeknél gyakran dokumentált, hogy a szomatikus funkciók fenntartása akár a szaporodási ráta csökkentése révén is biztosított, például táplálék szempontjából kihívást jelentő körülmények esetén (például: Gaillard et al., 2000; Therrien et al., 2008). Ez alapján a táplálék elérhetőség, túlélés és szaporodás kapcsolata is eltérhet az iteropár és az alacsony túlélést mutató szemelpár vonalak/populációk között. Habár néhány korábbi kutatásban is megjelenik ez a két mortalitási mintázat (Tomczyk et al., 2015; Yoshida et al., 2006), a fenti hipotézis tesztelésére különböző vonalak és populációk szélesebb körű bevonására lenne szükség a jövőben.

Mindent egybevetve, diszetációs munkám révén a regeneráció több aspektusával kapcsolatban is újabb összefüggéseket sikerült felfedezni, melyek további kutatási irányokat is segíthetnek. Megvizsgálva a nyeles hidrák ivaros és ivartalan módjait, őssejt készletét és fejregenerációs képességét, azt találtuk, hogy az ivaros szaporodás nagy fiziológiai költséggel bír (1. tanulmány). A csökkent regenerációs képességek és az őssejtek kimerülése alapján az ivaros szaporodás visszafordíthatatlan szaporodási stratégiának tűnik, mely az ivarsejt képzést helyezi előtérbe a szomatikus funkciók helyett. Az igen eltérő, szaporodási módtól függő életmenet döntések ideális model szervezetté teszik a nyeles hidrákat az öregedő és nem öregedő stratégiák tanulmányozására, akár fiziológia tekintetében is. Annak érdekében, hogy a limitált sejtszintű források szerepét a jövőben részletesen tisztázni tudjuk, több életmenet jelleg kialakításához szükséges, közös sejt készletet vizsgáló további kutatások indokoltak.

Az ivaros és ivartalan szaporodás őssejtekkel mutatott összefüggései későbbi vizsgálatunkban is szignifikánsnak mutatkoztak, azonban egy újabb tényezővel, az életkorhoz kapcsolódó mintázatok feltérképezését is megkezdtük (2. tanulmány). Egyazon hidra vonalon belül elvégzett kísérletünk eredményei azt sugallták, hogy az ivartalan szaporodás a regenerációval és az önfenntartással együtt fiatalabb életkorban priorizált, míg a nagyobb szomatikus funkciókat érintő költségekkel járó ivaros szaporodás későbbi életszakaszban kerül előtérbe, ivaros szaporodás indukálása esetén. A nyeles hidrák klón vonalon belül mutatott jelentős korfüggő plaszticitása rámutathat a laborban, klonális vagy parciálisan klonális fajokon végzett kutatások ismételhetőségének egy fejlesztési irányára. Az ivartalan szaporodás formái elterjedtek a modellszervezetek körében (például planáriák, vízibolhák, kerekesférgek, tengerirózsák és hidrák esetében), mely állatokat gyakran ivartalanul felszaporított, különböző korú egyedekből álló törzstenyészetekben tartják (Hughes, 1989 és a benne szereplő referenciák). Tanulmányunk arra utal, hogy azonos környezeti hatások eltérő szaporodási és forrásallokációs mintázatokat indukálhatnak nyeles hidra polipoknál életkoruktól függően. Következésképpen, ez a jelenség más klonális vagy parciálisan klonális szervezetnél is fennállhat, így a korra való kontrollálás más tudományos területeken is elősegítheti az ismételhetőség fokozását.

Végül, a fiziológiás regenerációval kapcsolatban, a táplálék elérhetőség vizsgálata révén próbáltunk fényt deríteni a nyeles hidrák ivaros szaporodást követő drasztikus túlélés csökkenésének hátterére (3. tanulmány). Mivel az elérhető táplálék mennyisége csak az ivarszervek számára volt hatással, a későbbi túlélésre nem, így nem egyértelmű, hogy a túlélésért felelős szomatikus funkciók fenntartásának gátoltsága a csökkent forrásallokációnak köszönhető-e. Meglepő módon azonban, ivaros szaporodás utáni degenerációt követően az állatok egy része képes volt regenerálódni, így felvetették egy újabb, iteropár szaporodási stratégia meglétét ennél a fajnál. A különböző vonalak között is markáns eltérések mutatkoztak mind az ivaros szaporodás indukálhatóságának, mind az egyedek ivaros szaporodás utáni túlélésének tekintetében, mely különbségek egy széleskörű populációs vizsgálat szükségességét vetítik előre.

Összességében elmondható, hogy a regeneráció mint fontos életmenet jelleg vizsgálata segíthet jobban megérteni az élőlények életmenet csereviszonyait, az eltérő öregedési mintázatok okait és ezek fiziológiai hátterét, mely vizsgálati célokra a nyeles hidrák igen alkalmasnak bizonyultak.

1.6. Összefoglalás

A regeneráció az élő szervezetek egyik alapvető sajátossága, helyreállító és megújulási folyamatok révén a sejtek, szövetek károsító és sérüléseket okozó tényezők elleni rezisztenciáját teszi lehetővé. Szerepe széleskörű, az egyedfejlődés, mintázat kialakítás, ivartalan szaporodás vagy az öregedés jelenségében is részt vesz. Az életmenet jellegek, így a regenerációs folyamatok is költséges mechanizmusok sorát követelik meg, ezért szükségszerűen alakulnak ki csereviszonyi hálózatok az életmenet komponensek között. A regeneráció számos szervezetnél része az ellentétes életmenet komponensek – mint a szaporodás és az önfenntartás - között kialakuló csereviszony kapcsolatoknak, azonban más önfenntartásért felelős életmenet jellegekkel való kapcsoltsága kevéssé ismert. Kutatásaim alanyai a rendkívüli regenerációs képességgel bíró nyeles hidrák (*Hydra oligactis*), melyek keretein belül többek között a regeneráció különböző önfenntartásért és különböző szaporodási módokért felelős életmenetjellegekkel közötti összefüggéseket vizsgáltam életkor és táplálékelérhetőség függvényében. Másfelől munkám során a csereviszonyok kialakulásának meghatározó tényezőjének, a belső, limitált forrásért való versengésnek a következményeit is kutattam. A nagy szöveti plaszticitással rendelkező állatok, így a édesvízi hidrák esetében is az őssejtek potenciálisan ilyen limitáló faktorként jelennek meg, ugyanis multi- vagy pluripotens őssejtekből származnak mind a szomatikus, mind az ivarsejtek. Ezen élettani háttérnek köszönhetően, ivaros szaporodás esetén a korlátozott őssejt forrásokért való versengés kihathat az egyedek regenerációs képességeire.

Eredményeink alapján alátámasztottuk az őssejtek mediátor szerepét a szomatikus és reproduktív funkciók kialakításában, szaporodási módok tekintetében pedig azt találtuk, hogy az ivaros szaporodás magasabb költséggel jár mint az ivartalan szaporodás, mind az őssejtek, mind a fejregeneráció tekintetében. A visszafordíthatatlannak tűnő ivaros szaporodás iránti elköteleződés mértéke azonban nagyban függhet az egyedek életkorától. Az ivartalan szaporodás a regenerációval és az önfenntartással együtt fiatalabb életkorban priorizált, míg a nagyobb, szomatikus funkciókat érintő költségekkel járó ivaros szaporodás későbbi életszakaszban kerül előtérbe. Az azonos körülmények között tartott egyedek életkor függő eltérő viselkedése más klonális vagy parciálisan klonális modellszervezetnél is fennállhat, ezért a korra való kontrollálás más tudományos területeken is elősegítheti a repetabilitást fokozását.

Munkám során a fiziológiás (fenntartó) regenerációra ható tényezőként az elérhető táplálék mennyiségét is vizsgáltuk, több nyeles hidra vonal bevonásával. Noha az ivarszervek számára szignifikáns hatással volt a táplálék mennyisége, a túlélésre nem volt hatással, így további vizsgálatok szükségesek a táplálékelérhetősg szerepének egyértelmű tisztázásához. A különböző hidra vonalak között azonban markáns eltérések mutatkoztak a túlélésének tekintetében, ugyanis meglepő módon az ivaros szaporodás utáni degenerációt követően az állatok nagy része képes volt regenerálódni, míg másik része - a korábbi tanulmányok eredményeinek megfelelően - elpusztult. Ezen markáns különbségek egy széleskörű populáció vizsgálat szükségességét vetítik előre. Összességében elmondható, hogy a regeneráció, mint fontos életmenet jelleg vizsgálata segíthet jobban megérteni az élőlények csereviszonyi hálózatának kapcsolatait, az eltérő öregedési mintázatok okait és ezek fiziológiai hátterét, mely vizsgálati célokra a nyeles hidrák igen alkalmasnak bizonyultak.

1.7. Az értekezés alapjául szolgáló tudományos közlemények

I Sebestyén, F., Barta, Z., & Tökölyi, J. (2018). Reproductive mode, stem cells and regeneration in a freshwater cnidarian with postreproductive senescence. Functional Ecology, 32(11), 2497-2508.

II Sebestyén, F., Miklós, M., Iván, K., & Tökölyi, J. (2020). Age-dependent plasticity in reproductive investment, regeneration capacity and survival in a partially clonal animal (*Hydra oligactis*). Journal of Animal Ecology, 89(10), 2246-2257.

III Tökölyi, J., Ősz, Z., Sebestyén, F., & Barta, Z. (2017). Resource allocation and post-reproductive degeneration in the freshwater cnidarian *Hydra oligactis* (Pallas, 1766). Zoology, 120, 110-116.

1.7.1. Személyes hozzájárulás (*) az értekezéshez tartozó tudományos közleményekhez

	1. tanulmány	2. tanulmány	3. tanulmány
Kutatás megtervezése	*	*	
Adatgyűjtés	*	*	*
Adatelemzés	*	*	
Kézirat elkészítése	*	*	*

1.8. Köszönetnyilvánítás

Ezúton is szeretném megköszöni témavezetőmnek, Tökölyi Jácintnak a sok évnyi készséges támogatását és segítségét, nélküle nem kezdtem volna bele. Hálával tartozom a labor és terepi munka hatékonnyá és élvezetessé tételeért a hidrás labor tagjainak, Miklós Máténak, Kozma Beatrixnak, Gergely Rékának, Nehéz Erzsébet Ágnesnek, Ngo Khanak, Iván Katalinnak,Tenkei Dávidnak és Ősz Zsófiának. Köszönöm tanszékvezetőnknek, Dr. Barta Zoltánnak az észrevételeit és támogatását, valamint az Evolúciós Állattani Tanszék munkatársainak, amiért jó munka légkört biztosítottak. Freytag Csongor fáradhatatlanul állt rendelkezésemre bármilyen kérdéssel, eszmefutattással és támogatással kapcsolatban, amiért hálám örökké üldözni fogja. A doktori munkám az Emberi Erőforrások Minisztériumnak Új Nemzeti Kiválóság Programjának (ÚNKP-18-3) támogatásával készült.

2. Regeneration and senescence in freshwater hydras: life history decisions and their physiological background

2.1. Introduction

2.1.1. Definition of regeneration and its relation to stem cells and asexual reproduction

Wounds, environmental factors, diseases, or stress pose a constant threat to multicellular organisms. To cope with the ensuing damages, two main processes have evolved: scar formation and regeneration (Stocum, 2018). Scar formation is completed without the replacement of the lost body part, and preserves the integrity of the given area (Stocum, 2018). In contrast, regeneration includes restorative processes against the negative impacts (Stocum, 2018). According to the prevalent definition, regeneration implies the restoration of lost body parts, which can refer to regeneration at a cellular, tissue, organ or whole-body level (Bely & Nyberg, 2010).

The scientific history of regeneration can provide insights to the formation of its definition. The beginning of regeneration research roots in the 18th century, when René-Antoine Ferchault de Réaumur observed limb regeneration on freshwater crayfish for the first time in 1712 (Ratcliff, 2012). As a result of Réaumur's detailed description of leg and claw regeneration, scientific interest in the phenomenon of regeneration intensified. Abraham Trebley improved Réaumur's techniques and performed experiments on hydras (Esposito, 2013), providing a basis to biological experimentation and hypothesis testing (Dinsmore, 1991). The beginning of regeneration research in vertebrates is credited to Lazzaro Spallanzani, the third leading scientist of the era, who examined tadpoles in the late 18th century (Capanna, 1999).

However, the definition of regeneration became controversial in the 19th-20th century, when two views formed. August Weismann (1893) defined regeneration as the result of cellular renewal processes, while, based on his empirical measurements, Thomas Hunt Morgan (1901) considered the definition more complex and distinguished two main types of regeneration. According to his categorization, if regeneration requires cell division and blastema formation from the divided cell (blastema: undifferentiated cell aggregation at the wound site, which generates the regenerating body part (Bely & Nyberg, 2010)), it is called *epimorphosis*, but if regeneration is proceeded via tissue rearrangement, it is named to *morphallaxis* (Morgan, 1901). He differentiated three further cases of epimorphosis, depending on whether the newly created organ was identical to the previous one ('homomorphosis'), it was smaller compared to the replaced organ ('meromorphosis') or differed morphologically and physiologically from the former one ('heteromorphosis') (Esposito, 2013). The discussions about concepts described well the views of that era. Weismann thought that animal regeneration evolved contingently, and a consequence of adaptation due to natural selection. Conversely, Morgan posited that living organisms had regeneration universally and the ability to regenerate was a general feature but appeared at different degrees (Esposito, 2013). Although both definitions were empirically supportable by other researchers' work (Esposito, 2013), the clarification of this debate was made more difficult by the lack of modern research technologies.

Despite continuous research, our knowledge is still not complete, therefore we cannot undoubtably answer the question whether regeneration is an adaptive or an inherent trait up to this day. Nevertheless, results suggest that regeneration is one of the fundamental features of Eumetazoa, connected to embryonal development and its loss is linked to the appearance of another, pleiotropic trait that is increases more the lifetime fitness (Goss, 1992). Based on a newer study, there is also evolutionary evidence to the emergence of regeneration in certain body parts (Zattara et al., 2019). The complexity of the phenomenon likely contributed to the fact that the definition of regeneration became more and more widely extended rather than becoming more specific during its 300 years old history. **This tendency is also reflected by the fact that currently several restorative processes are accepted as regeneration resulted by either physiological cyclicity (e.g. blood and skin renewal) or injuries, moreover, even a branch of medical practice is defined as „regenerative medicine”** (Sánchez Alvarado, 2012).

Intensive research of regeneration helps us to specify long used definitions and discover new, general patterns. Morgan’s epimorphosis and morphallaxis terminology remained widely in use after his work. However, the differentiation of the two types is still controversial (Agata et al., 2007; Kostyuchenko & Kozin, 2020; Maden, 1981). Regeneration in planarians can be a good example to demonstrate the equivocal distinction between the two categories. Following an incision on planarians, there is an epimorphotic blastema formation perceptible to the naked eye, but a tissue rearrangement in the stump is also observable, which process is classified as morphallaxis. Morgan himself described both phenomena, but later on, other works also confirmed that processes, which are characteristic to both epimorphosis and morphallaxis, are present in planarian regeneration (Rink, 2018). The major, cell division-related criteria of the two concepts also seem to have been overthrown: cell division only conditionally results in blastema formation during epimorphosis, furthermore cell division can occur during morphallaxis as well (Pellettieri, 2019). Due to the improvement and headway of molecular and cellular methods, newer results support also that tissue rearrangement (i.e. morphallaxis) is required for the initiation, transitional stages and termination of epimorphosis in case of annelids (Kostyuchenko & Kozin, 2020). Because of the difficult classification of the two regeneration types, potentially there is a confusion in the literature (Agata et al., 2003) that is true for hydras as well (Chera et al., 2009). Therefore, I better don’t refer to them in my dissertation.

However, the advancement of regeneration research made possible not just the rethinking of long-term definitions, but brought up new aspects like the role of stem cells (Gurley & Alvarado, 2019). The important contribution of **stem cells** to animal regeneration is widely known, for example in sponges, flatworms or even mammals (Funayama, 2018; Gurley & Alvarado, 2019). Stem cells are characterized by continuous renewal and are able to produce new, differentiated cells (Adler & Alvarado, 2015). Typically, they are in a resting state or have slow cell cycle, but an injury can activate numerous functions (Bergmann & Steller, 2010). Through establishing new cells, stem cells can be a major source for injury-induced tissue loss (beside de-, re- and transdifferentiation of other cell types (Sugimoto et al., 2011)), participate in blastema formation and induce several signalling pathways (Gurley & Alvarado, 2019). In some coral species, stem cells are involved in development of a blastema-like structure and its transdifferentiation (Funayama, 2018). In the case of *Drosophila*, the division of gastrointestinal stem cells enables the regeneration of the whole middle intestine section during 2-3 days (Bergmann & Steller, 2010). In planarians, the division of stem cells (neoblasts) in their body increases after 6 hours following amputation, and after 48 hours they make whole body regeneration possible (Ricci & Srivastava, 2018). Muscle tissue of mammals typically contains stem cells (satellite cells), in which proliferation is activated by damages and following differentiation they participate in the recovery of muscle fibers (Tanaka & Reddien, 2011). Regeneration is primarily dependent on tissue-specific stem cells in mammals (Yun, 2015), including humans as well (Carlson, 2011). Although stem cells can appear in different contexts in the studied organisms, their role is critical in numerous cases (Gurley & Alvarado, 2019).

Regeneration is related to another important phenomenon, **asexual reproduction**, which is a key biological trait in my thesis. A possible evolutionary origin of regeneration is traceable to asexual reproduction (Alvarado, 2000). If we examine the example of fission in the ciliate *Bursaria* for instance, we can ascertain that the only difference between its fission and regeneration is in the activating stimuli: fission requires unfavourable conditions while regeneration is triggered by any injury (Alvarado, 2000). Dedifferentiation at the beginning of the process, then redifferentiation of two cell pieces to new individuals take place in the same way both in regeneration and fission (Alvarado, 2000). Research investigating annelids confirmed that the epitope and protein patterns of regeneration and fission can have shared participants (Martinez et al., 2005). Furthermore, a likely common evolution of the two phenomena is supported by an evolutionary model (Zattara & Bely, 2016). Signalling pathway during head regeneration and budding is also proved to be similar (Javois et al., 1986). Parallel patterns to asexual reproduction found in many animals, lend support to the theory that the evolution of regeneration might begin with the appearance of asexual reproduction mechanisms triggered by injuries (Alvarado, 2000). Despite the similarities, Bely & Nyberg (2010) think that the unique stimuli, the presence of developmental features specific to regeneration (for instance characteristic gene expression patterns), together with the not completely concordant phylogenetic appearance of the two traits suggest that regeneration should be viewed as an evolutionarily independent trait.

Interestingly, asexual reproduction has been drescribed as playing role in the evolutionary origin for stem cells as well (Agata et al., 2006). Keeping at the example of fission in planarians, we know that the regeneration of missing body parts after fission is done with the involvement of stem cells (Agata et al., 2006). The presence of stem cells, their involvement in asexual regeneration and the high ability for regeneration is commonly described from sponges to chordates, therefore this observation also contributed to the assumption that stem cells evolved as a component of asexual reproduction (Agata et al., 2006). Examining the phylogenetic appearance of asexual reproduction, it is noticable that it is a widespread reproductive mode and a shared character down to choanoflagellates (Alvarado, 2000; Sköld et al., 2009). In most studied taxa, totipotent or at least pluripotent stem cells or flexible adult cells participate in the process of asexual reproduction, reflecting to the presence of high cellular and tissue plasticity (Sköld et al., 2009). But this raises the questionof how the three features (regeneration, stem cells and asexual reproduction) are evolutionarily connected to each other. Does asexual reproduction explain the close relation between regeneration and stem cells? What is responsible for the non-uniform phylogenetic distribution of regeneration? What are the factors which inhibit or inhibit regeneration ability in some species and individuals?

2.1.2. Regeneration as a life history trait and its costs related to stem cells To answer these more general questions we should inspect regeneration as a *life history trait*, i.e., as a trait that is functionally associated with reproduction and survival. In order to do this, it is important to consider that beside its unambiguous benefits, regeneration has many costs as well. Such a disadvantage can be i) the existence of transitional stages (a not fully regenerated body part can be more detrimental than its loss to the animal) ii) cost of accurate regeneration (if the regenerated body part is not completely equivalent or distorted relative to the original) iii) energy and resource allocation cost of regeneration (Bely & Nyberg, 2010). The energy cost of regeneration has been shown in many species, hence we know for instance that the standard metabolic rate increases with 36% during tail regeneration in lizards (*Liolaemus belli*, Naya et al., 2007) and some other lizzards can channel even more than half of their complete energy to tail regeneration (in *Gerrhonotus multicarinatus*, (Vitt et al., 1977). In *Parabuthus transvaalicus* scorpions, the metabolic rate increases with 39% during venom store regeneration (Nisani et al., 2007), and in echinoderms (sea lilies, brittle stars, sea stars) the main energy cost originates from reduced food intake ability during arm regeneration (Lawrence, 2010). Consequently, via its energy and resource allocation cost, regeneration can affect several life history traits of organisms. This is an exceedingly significant feature, since the life history of an individual covers its lifetime from birth to death, including the age- and state-dependent patterns of sexual maturity, reproduction, survival and death (Flatt & Heyland, 2011). Therefore the costs of regeneration influences reproductive success during lifespan by its effects on other life history traits.

Resources used by life history traits are limited, hence negative linkages (i.e. trade-offs) between them inevitably occur (Flatt & Heyland, 2011). Limiting resources can be macro- and micronutrients (Catoni et al., 2008; Cotter et al., 2011; Zera & Zhao, 2006) or metabolic reserves (Ellers, 1995), but any substance can be a subject of trade-offs, if it is required by multiple life history traits and if it can be depleted. In animals with high tissue plasticity (e.g., sponges, corals, cnidarians, and flatworms) the availability of stem cells can be such a finite resource (Rinkevich, 1996). The body of these animals consists of stem cells at a high proportion, which on the one hand can be responsible for shaping a wide variety of traits related to survival, like growth, food capturing stinging cell production, and their role in regeneration is also important as described above (Gold & Jacobs, 2013; Henry & Hart, 2005). On the other hand, it is also known that these stem cells can be responsible for the production of sexual and asexual reproductive cells (e.g. in sponges: (Simpson, 1984), in cnidarians: (Gahan et al., 2016), in planarians: (Newmark et al., 2008)). Based on the shared and finite stem cell pool, it is theoretically expected that an increased investment into reproduction incurs some physiological cost and results in a decrease of stem cell numbers available for differentiation into cells serving somatic functions such as regeneration (Henry & Hart, 2005; Rinkevich, 1996).

The involvement of regeneration in trade-offs is unambiguous, however several related aspects are less or unknown. I extended our knowledge about some aspects by studing regeneration related to stem cell pool, sexual and asexual reproductive modes and age, furthermore, examined resource allocation between sexual reproduction accompanied with intensive ageing and survival.

2.1.3. Differences in regeneration according to reproductive modes

The trade-off between reproduction and self-maintenance can be significantly influenced by the mode of reproduction, namely sexual or asexual modes as well. Although sexual reproduction has clear advantages in continuously changing unpredictable environments due to producing offspring with recombined genotype, it also has disadvantages as the "two-fold cost of males" and genome attenuation (Lehtonen et al., 2012). In case of asexual reproduction, these disadvantages do not arise, hence it can be evolutionarily favourable in certain circumstances (Crow, 1994). The reproductive value of offspring can play an important role in which reproductive mode is more successful, due to the survival probability of the offspring being different in given circumstances. If the sexual and asexual offpring's reproductive values are different, the amount of resources invested into reproduction should also differ, and indeed, this is commonly described in animals (e.g. tunicatas: (Yund et al., 1997), aphids: (Nespolo et al., 2009), *Daphnia*: (Innes & Singleton, 2000), hydra: (Kaliszewicz, 2011)). At the same time, we know much less about the physiological consequences of differential investment of resources depending on reproductive modes, as well as how animal regeneration and other self-maintenance traits are affected by this. To address the major predictions of life history theory about the reproduction–survival trade-off, studies investigating the mediator role of stem cells are especially necessary, which is a central topic of the research in Study 1.

2.1.4. Age-dependent changes

Besides the limiting stem cell pool, age and life stage are also possible factors that influence regeneration and drive phenotypic plasticity (Fischer et al., 2014). Age is one of the factors belonging to the organisms' internal state, correlates with condition, reproduction and survival (McNamara & Houston, 1996). Via information acquisition, age also highly contributes to decision making in an unpredictably changing environment (Fischer et al., 2014). Generally, the rate of restoration and regeneration of somatic damages is higher at a younger age, decreases after maturation and completely stops before the maximum expected lifespan (Cichoń & Kozłowski, 2000). Considering that regeneration is related to other traits due to the resource limitation, in order to understand its regulation, we should examine the sexual and asexual reproductive patterns as well. It is often true to both determinate and indeterminate growers that their fecundity increases with age, until their prime-age and then fecundity gradually decreases with age in the majority of determinate species (Clutton-Brock, 1984; Martin, 1995). In clonal and partially clonal animals (species capable of both sexual and asexual reproduction), however, age-dependent patterns can be much more complex and can differ from what is observed in non-clonal organisms (Clutton-Brock, 1984; Engen & Saether, 1994; Stearns, 1992), hence their integration into life history models involving self-maintenance is complicated (Glazier & Calow, 1992). According to conventional life history predictions, organisms favour growth at first, then growth stops and they start reproduction (Cichoń, 1997). In spite of this, research results of clonal animals show that the combination of reproduction at early life stages and resuming growth later is also possible, if the individuals are in good condition (Glazier & Calow, 1992). In partially clonal animals, another important decision is to switch from asexual to sexual reproduction, the timing of which can be flexible and might be triggered only when clonal reproduction is limited by exogenous factors (Burke & Bonduriansky, 2018; Gerber et al., 2018; Harvell & Grosberg, 1988).

While the theoretical differences between sexual and asexual reproduction are relatively well known (reviewed for example in: Meirmans et al., 2012; Neiman et al., 2014), reproduction *in parallel with* regeneration and other traits responsible for self-maintenance are much less studied, especially in reference to age-dependent changes. Regeneration also shows differences in relation to sexual and asexual reproductive modes, which is usually characterized with higher regeneration capability in asexual individuals (for example in case of a New Zealand freshwater snail species (*Potamopyrgus antipodarum*) (Krois et al., 2013) or in mole salamanders (Saccucci et al., 2016). It is also apparent that unusual patterns in age-dependent changes in regeneration are also possible, hence regeneration in young sponges can be completely blocked (likely due to the intensive involvement of stem cells in growth (Simpson, 1984)) and in certain partially clonal species does not decrease with age (Bodnar & Coffman, 2016; Yun, 2015). Asexual individuals are generally characterized with high self-maintenance traits (Sköld & Obst, 2011), but at the same time a decrease in regeneration (Meesters & Bak, 1995), survival (Orive, 1995) and DNA repairing enzyme (e.g. telomerase) activity (Sköld et al., 2011) with age is also observed. As the non-conventional examples demonstrate above, we are far from a complete understanding of age-dependent changes in regeneration. We know particularly little about resource allocation patterns according to reproductive modes and regeneration (or other traits responsible for self-maintenance) in clonal and partially clonal animals. My research in Study 2 serves to broaden our knowledge in this topic.

2.1.5. Relation of ageing and regeneration

A wide variety of regeneration processes can relate to not just age-dependent changes, but to the phenomenon of senescence (or ageing) as well. Most evolutionary biologists define aging as an age-dependent or age-progressive decline in intrinsic physiological function, leading to an increase in age-specific mortality rate (i.e., a decrease in survival rate) and a decrease in age-specific reproductive rate. So far in my dissertation I primarily focused on the context of regeneration referring to the injury induced scenario, but based on Stocum’s (2018) description, another, ageing-related type of regeneration is called *physiological or maintenance regeneration*. During physiological regeneration, the replacement of old cells with new ones (called cell turnover) is a homeostatic process, which maintains and ensures the normal tissue structure and function (Stocum, 2018). This hidden process can be found in all multicellular organisms at the level of epidermis, blood and gastric cell replacement for instance (Kostyuchenko et al., 2016). Beside injury-induced regeneration, ageing mechanisms influence this physiological regeneration as well.

The effect of ageing on physiological regeneration is fundamentally a consequence of upset cell division regulation (Seifert & Voss, 2013). A wide range of processes responsible for ageing is known, among others it can happen due to increasing DNA damage, telomer erosion, dysregulation of gene expression and protein functions, disruption of cellular matrix and organelles, deteriorated intercellular communication, oxidative stress or metabolic disruption (McHugh & Gil, 2018; Sousounis et al., 2014). These processes can cause physiological damage in several ways and are able to appear as factors inducing cellular senescence (Chiche et al., 2020). This stable, cell cycle inhibiting state of cellular senescence appears at the end of replicative lifespan in diploid cells, indicating a hallmark for ageing (Chiche et al., 2020). The division ability of senescent cells are restorable with (partial) reprogramming, but there is a higher risk for tumour development in damaged cells, which risk is – among other factors – likely underlying the evolution of mechanisms that accumulate the old damaged cell accumulation in the body (Chiche et al., 2020). As the example of cellular senescence and most studies until now confirms, the decrease in physiological regeneration processes in parallel with ageing is a general phenomenon (Yun, 2015). Accordingly, some theories suppose that ageing is a result of decline in regeneration ability and maintenance of properly functioning cells and tissues (Sharpless & DePinho, 2004). This concept is also supported by the existence of organisms having remarkable regeneration capabilities and lacking measurable functional decline, which are the sign of negligible senescence (Finch, 1994). Moreover, in species with high reparative and regeneration capabilities, even negative senescence (i.e. decreasing mortality and increasing fecundity with age) can occur, usually paired with such characteristics as continuous oogenesis, high amount of stem cells, lack of somatic and germline differentiation or the ability to asexual reproduction (Vaupel et al., 2004).

2.1.6. Role of resource allocation in ageing

In order to understand the radically different forms of senescence such as negative senescence, we have to examine their fundamental causes (Baudisch & Vaupel, 2012). Allocation theory is a good framework for this approach, as this theory aims to unravel how resource limitation influences life history traits (Baudisch & Vaupel, 2012). According to this theory, evolutionary fitness is based on the optimal resource allocation between competing processes supporting survival and/or reproduction (Davison et al., 2014). Hence, each decision (strategic choice without cognition or will) has a consequence affecting survival, reproduction or growth via trade-offs (Davison et al., 2014). Although senescence theories acknowledge the presence of allocational trade-offs, their fundamental importance is neglected and senescence theories focus on the decreasing effect of selection with age following maturity (i.e. selection shadow) (Baudisch & Vaupel, 2012). This might explain why we know little about the selection forces favouring or disfavouring senescent and non-senescent patterns (Baudisch & Vaupel, 2012). Such a selection force can be the minimum required metabolic investment to survive or the availability of resources (Davison et al., 2014). The availability and limitation of resources can be related to various changes in survival or reproduction. A typical example to this is the lifespan elongating effect of dietary and caloric restriction, which is supported by studies in mice, *Drosophila* and *Caenorhabditis elegans* (reviewed in: (Kennedy et al., 2007)). However, insufficient food can be a cue for an opposite phenomenon, since during terminal investment survival is reduced, while energy investment is focused on increasing reproduction in a final effort

(Clutton-Brock, 1984; Fischer et al., 2009). Nevertheless, animals often give different responses to the same environmental impact. For instance, the increased investment into repairing and maintenance mechanisms reduces fecundity in some species, or has a weak effect in others, while reduces present and increases future fecundity in yet another species (Baudisch, 2012). The investigation of these species-specific diversity can help to understand the evolution of senescent and non-senescent ageing patterns, therefore they should receive higher research interest (Baudisch & Vaupel, 2012). Species of the genus *Hydra* are good candidates for the investigation of this diversity. The members of this genus are generally thought to have negligible senescence (Dańko et al., 2015), but one species (*Hydra oligactis*) shows intensive degeneration and reduced survival following sexual reproduction, which are characteristics of senescence (Yoshida et al., 2006). For this reason, we studied this senescent species by investigating resource allocation, reproduction and survival in parallel and according to age (Study 3).

2.1.7. Freswater hydras as model organisms

Hydras earned the “immortal” distinction owing to their capacity of constant fertility coupled with low mortality and this distinction raised research interest in hydras in the past decade (Martínez, 1998; Schaible et al., 2015). Their research began with a regeneration study by Abraham Trembley in 1744. Trembely’s question was to decide about green hydras (*Hydra viridissima*) whether they are plants or animals. He thought if they could regenerate following a bisection, they were plants, and if the regeneration failed, they were animals. His experiment finished with the regeneration of both halves, but in the meantime, he could observe prey capturing by hydra individuals, therefore - correctly - he considered hydras as animals (Lenhoff & Lenhoff, 1988). Since then, our knowledge has broadened with a great amount of information during the past 270 years. Nowadays it is known that a few dozens, small sized, predatory species belong to the genus *Hydra* (Kawaida et al., 2010). Of these, there are 5 European species (Schuchert, 2010) and mostly two species, common hydras (*Hydra vulgaris*) and brown hydras (*Hydra oligactis*) are under rather intense research. A common characteristic of hydras is that their body contains stem cell at a high rate: endothelial, epithelial and interstitial stem cells, and the latters are responsible for production of nerve, stinging, gland and germ cells and thus have multipotency (Nishimiya-Fujisawa & Kobayashi, 2012). Through the division of stem cells, hydras constantly exchange their body proceeding from their trunk forward to their tentacles, thereby they can ensure the renewal and intensive physiological regeneration of their body (Campbell, 1967a, 1967b). Their ability for asexual reproduction further enhances the presence of high tissue plasticity, since it also requires the constant growth and cell turnover (Holstein et al., 2003). The intensive stem cell division, lack of complete separation of germline and somatic stem cells and ability for asexual reproduction all likely facilitate the negligible senescence observed in *Hydra vulgaris* and *Hydra magnipapillata* (Martínez & Bridge, 2012; Orive, 1995), which can be also enhanced by their regeneration capability involving stem cell activity (Chera et al., 2009; Govindasamy et al., 2014). Beside their physiological regeneration, hydras also have remarkable injury induced regeneration abilities, since they can regenerate their whole body from a piece of tissue as less as 1% of their original body size (Shimizu et al., 1993). Researches of hydras so far studied mainly the physiological, developmental and molecular aspects of this phenomenon (Bode, 2003;

Kemmner & Chica Schaller, 1984; Tardent, 1974) and we understand much less the regeneration in a life history context, therefore I focused on this area in my work.

Regeneration is a plastic trait in hydras as well, and well demonstrated in the brown hydra (*Hydra oligactis*), which shows delayed or arrested regeneration in sexual individuals (Galliot et al., 2018; Tomczyk et al., 2017). The loss of regeneration ability is likely explained by their reproductive mode: they switch from asexual to sexual reproduction to the stimulus of temperature drop in autumn, in order to produce eggs resistant to the harsh winter environment at a high number, compared with other *Hydra* species (Reisa, 1973; Schuchert, 2010). Differently from other species, significant changes can be observed in brown hydras during and following sexual reproduction. Their interstitial stem cell number strongly decreases with increasing reproductive cell number, then following sexual reproduction, their body degenerates and they most probably die (Tardent, 1974; Yoshida et al., 2006). Because of these drastic changes, senescence is thought to be a consequence of sexual reproduction“gametic crisis”, which can be caused by the exhaustion of their stem cells during the intensive differentiation to germ cells, and hence their ability to maintain somatic functions is probably inhibited (Bosch, 2009; Brien, 1966; Tardent, 1974). Although, we have to notice the fact that these studies were based on a few laboratory strains, without investigating the importance of access to food or age. Furthermore, we do not understand the reproductive strategies in natural populations and the regulatory roles of competition for cellular resources therein, which questions are raised and studied in Study 1, 2 and 3. Considering that the investigation of senescent and non-senescent pathways is possible in closely related hydra species, and even within species in brown hydra. Therefore they can serve as ideal model organisms to study regeneration, senescence and their consequences related to life history traits, which are fundamental topics in my dissertation.

2.2. Objectives

2.2.1. Role of stem cell pool and reproductive modes (Study 1)

Due to their multifunctionality, stem cells can play major roles in traits belonging to different and opposing life history components at the same time. Reproduction can vigorously use stem cell resources, resulting in trade-offs with self-maintenance traits like regeneration, which also relies on stem cells. However, the differences between sexual and asexual reproduction can appear in terms of their stem cell requirements as well, which can have an effect on other traits. **In this part of my dissertation, I set the objective of investigate the relation between sexual and asexual reproduction, availability of stem cells and its dependent traits as regeneration and stinging cell number.** The main questions of our research in the light of this framework are listed below:

(1) Does head regeneration ability differ with reproductive modes?

(2) Are the changes in head regeneration ability linked to changes in the number of stem cells?

(3) Are there any difference in the number of stem cells and stinging cells in different reproductive modes (nonreproductive, asexual, sexual)?

(4) Is there a correlation between the number of reproductive cells, stem cells and stinging cells in animals derived from their natural habitat?

We used brown hydras (*Hydra oligactis*) as model organisms, which we collected from their natural habitat in order to have an insight to natural populations. Based on former studies, it is known about brown hydras that part of the population is able to switch from asexual to sexual reproduction in autumn, followed by the degeneration and death of the individuals. The winter survival of sexual offspring is presumably higher since the resting eggs are resistant to freezing.

According to our predictions(i) sexual individuals should invest in reproduction at a higher rate (ii) and the excess investment should result in reduced stem cell pool (iii) due to the mediator role of stem cells, the availability of resources for self-maintenance traits as regeneration and production of stinging cells is expectedly also reduced, compared to the asexually reproducing individuals. Furtherfore, (iv) in case of the existence of trade-offs among reproduction and self-maintenance, we can predict a correlation between the number of reproductive cells and stem cells as well.

2.2.2. Age-dependent plasticity in reproduction and self-maintenance (Study 2)

Fundamental life history traits often show age- and life stage dependent plasticity. Due to the asexual reproductive mode, age-dependent life history decisions and priorities can be quite complex in clonal or partially clonal animals, showing regeneration and reproduction patterns differing from what could be expected based on conventional models. In case of partially clonal animals, the induction and timing of switching from asexual to sexual reproduction is an additional important life history decision. While the differences in sexual and asexual reproduction are better studied, we know much less about what alterations in regeneration and self-maintenance are paired with age- and reproductive mode dependent resource allocation patterns in clonal and partially clonal animals. **For this reason, we investigated age-dependent plasticity in traits related to reproduction (gametogenesis and asexual reproduction) and self-maintenance (head regeneration and survival) in brown hydras, by using temperature lowering to induce sexual reproduction.** The purpose of our experiment was to answer the following main questions:

(1) Do sexual and asexual reproductions change in parallel with the investment in regeneration and survival in younger and older individuals?

(2) How do regeneration ability and number of stem cells change in the two age groups, following sexual reproduction inducing temperature drop?

(3) Is there a difference in the timing (start) and intensity (number of sexual organs) of sexual reproduction in younger and older individuals?

(4) Do the age-dependent, phenotypic changes in regeneration and reproduction support the measured quantitative values of cell types (stem cells, reproductive cells)?

If fecundity increases with age but somatic maintenance decreases according to general life history theories, then the numbers of reproductive organs and reproductive cells should be prospectively higher in older animals, while their regeneration ability, survival and interstitial stem cell number should be lower compared to younger animals. Oppositely, it is also possible that older animals are able to maintain traits belonging to both life history components, as a consequence of resource acquisition during their longer lifespan. Based on previous observations, the prioritization of asexual reproduction is rather expected in the younger age group, but opposite values are also possible due to the better condition of older individuals.

2.2.3. Resource allocation between sexual reproduction and survival in the background of ageing (Study 3)

Generally, a good indicator for senescence, is that physiological regeneration also declines, which refer to the renewal ability of cells and tissues influencing survival. This is often observed in the life stage following sexual reproduction, supposedly because of resource allocation as one of its main reasons. Via the trade-offs based on resource limitation, life history decisions influence the two major life history components, namely reproduction and self-maintenance. However, the consequences of life history decisions can be varying, depending on the availability of resources. **In this part of my dissertation work, we examined the effect of food availability on sexual reproduction and survival in brown hydras showing intense postreproductive degeneration and ageing**. In our experiment, we aimed to answer these central questions:

(1) How can food availability (at four levels) influence the occurrence of sexual reproduction, number of sexual organs and survival rate?
(2) Are the extent of sexual reproduction and later survival rate in correspondence?
(3) Are there any differences among different strains derived from their natural habitat in terms of propensity to sexual reproduction, extent of sexual reproduction and the following survival rate?

If two life functions (reproduction and self-maintenance) are important at the same scale, we expect similar reduction therein in case of low food levels. However, if traits related to the same life function are not equivalent, the prioritization of one or the other is presumable. Extreme outcomes of this phenomenon occur in case of dietary restriction (reduced fecundity and increased survival) and terminal investment (intensive fecundity and reduced survival).

2.3. Materials and methods

2.3.1. Role of stem cell pool and reproductive modes (Study 1)

We collected brown hydras from their natural habitat (an oxbow lake near Tiszadorogma) on four occasions, in the reproductive period during autumn and fall. We gathered individuals from submerged vegetation, placed them into Eppendorf tubes, then brought them back to the laboratory in a cooling box. The following day, I began the head regeneration experiment on a randomized half of the individuals. I cut the head region of the animals as a first step, then I measured regeneration with a binary code system, based upon the presence or absence of newly emerged tentacles after four days (presence of tentacles = regeneration). We kept animals in climate chambers during this experiment, according to temperatures measured in their natural habitat. Another subset of the animals participated in cellular measurements. We macerated the animals following the standard procedure on the first day after collection, and counted the following cell types later: epithelial cells, interstitial stem cells, nematoblast (stinging cells) cell clusters, and reproductive cells (sperm precursor clusters in males and nurse cells in females). For statistical analyses, I used Generalized Linear Mixed-effects Models (GLMMs), with error distributions according to the data (and performed in a Bayesian environment if it was needed).

2.3.2. Age-dependent plasticity in reproduction and self-maintenance (Study 2)

The male and female strains used in this experiment originated from an oxbow lake near Tiszadorogma, then kept in strain cultures for one year prior to the experiment. We kept the animals in standard conditions, constant temperature at 18 °C in a climate chamber. We used freshly detached buds for the experiment. As an experimental treatment, we reduced the temperature to 7 °C, 3 or 24 days after the beginning of the experiment. We examined the animals several times a week, to record their asexual reproduction rate and number of sexual organs. I measured regeneration weekly for two months at a subgroup of animals. I binary coded the emergence of new tentacles following decapitation (yes/no). We also performed cellular measurements approximately weekly for two months, counting epithelial cells, interstitial stem cells, sperm precursor cell clusters and nurse cells. We kept the experimental animals for 5 months recording death events, then binary coding survival. To analyze our data, I used GLMMS with proper error distributions, then performed Likelihood Ratio Tests (LRTs).

2.3.3. Resource allocation between sexual reproduction and survival in the background of ageing (Study 3)

The used brown hydras strains originated from the oxbow lake near Tiszadorogma. Firstly, we collected 10 animals, then kept them under standard conditions at 20 °C for two months for asexual propagation. We fed the animals three times a week during the experiment, according to 4 treatments giving different amount of food as much as 3, 6, 10 or 20 µl *Artemia* at feeding occasions. Two weeks after beginning the experiment, we reduced the temperature to 7 °C to induce sexual reproduction. In order to measure sexual reproduction rate, we counted sexual organs (detached eggs in females and testes in males). We recorded the death of animals if they disintegrated or disappeared. We were especially careful to retain animals shrank to a really small size. We kept the animals for 150 days, until most individuals were dead, or regenerated after sexual reproduction and produced at least one asexual offspring. For the statistical analyses, we used Generalized Linear Models (GLMs) or GLMMs, followed by Likelihood Ratio Tests with Poisson or Binomial error distributions.

2.4. Results

2.4.1. Role of stem cell pool and reproductive modes (Study 1)

- Sexual individuals had reduced regeneration ability and stinging and interstitial stem cell pool, compared to asexually reproducing polyps of brown hydras. We found no difference between asexual and nonreproductive individuals.
- The number of germ cells measured in sexual individuals negatively correlated with the number of interstitial stem cells and stinging cells.
- Our results of regeneration ability and cell composition confirms the differing physiological costs of sexual and asexual reproduction.
- Our results support the interpretation of regeneration as a life history trait and the theory, according to which the competition for stem cell resources has a mediator role in life history decisions.
- We confirmed in animals collected from a natural population the previously observed cell types patterns about declining stinging and stem cell numbers following sexual reproduction, yet known from only a few, long-term laboratory kept strains.

2.4.2. Age-dependent plasticity in reproduction and self-maintenance (Study 2)

- We confirmed the presence of age-dependent plasticity in all of our measured traits (head regeneration rate, asexual reproduction rate, number of sexual organs, start of sexual reproduction, survival, number of reproductive cells, number of stem cells)
- Younger individuals had higher asexual reproduction, while older animals showed more intense gametogenesis and reproductive cell production in our experiment.
- In parallel with the differences in reproduction, we observed age-dependent differences in self-maintenance traits as well: older individuals reproducting rather sexually had lower head regeneration ability, survival and stem cell number.
- Besides the impact of plasticity, the age-dependent changes in head regeneration rate and stem cell number also imply ontogenetic effects,because younger individuals initiated sexual reproduction in a delay similar to the age difference between the younger and the older group.

2.4.3. Resource allocation between sexual reproduction and survival in the background of ageing (Study 3)

- Sexual reproduction was followed by reduced survival in brown hydras, but in spite of previous studies, a significant proportion of animals regenerated following degeneration after sexual reproduction and they even started asexual reproduction. This is a unique life history in animals and a unique consequence of trade-off among reproduction and survival.
- Food amount didn’t have an impact on the start of sexual reproduction, but had an effect on the number of sexual organs, resulting in sexual organ production at a high rate.

- Survival rate was not influenced by the amount of available food, suggesting that survival is a conserved trait, and its maintenance is important even at a low food level.
- There was no linkage between the number of sexual organs and survival at individual levels.
- We observed a difference between 10 brown hydra strains involved in our experiment in the aspects of the propensity to sexual reproduction, number of sexual organs and survival. We also found strains with lacking or low propensity to initiate sexual reproduction.

2.5. Discussion

In my dissertation work, I studied regeneration as a widespread life history trait, investigating it in parallel with traits related to reproduction and somatic maintenance. I studied regeneration and related traits in freshwater hydras, in accordance with three main factors: (i) stem cell resources, (ii) age and (iii) food availability. We discovered strong relations between regeneration and traits related to opposing life history components in all cases. Furthermore we described differences between sexual and asexual reproductive modes as well. I discuss the possible interpretations of our major results down below, according to the main contexts of our research.

Regarding stem cells, we found marked, depending on reproductive modes in hydras collected from their natural habitat (Study 3). Compared to the nonreproductive group, sexual individuals showed lower head regeneration ability, reduced stem cell and food capturing stinging cell pool, while asexuals did not differ. The evolutionary cause of reduced somatic functions (regeneration, stem cells, feeding) related to sexual reproduction might be an effect of selection, prioritizing young or current reproduction instead of long-term survival. Several evolutionary theories of ageing say that mutations deleterious to survival can spread in a population, if the externally caused mortality is high (mutation accommodation theory: Medawar, 1952, antagonistic pleiotropy theory: Williams, 1957, disposable soma theory: Kirkwood & Rose, 1991). The potentially high mortality risk is true for hydras living in the temperate zone, because the onset of cold weather and frost is especially dangerous in shallow water bodies. In these cases, the production of frost resistant resting eggs can have a higher reproductive value than asexual offsprings, therefore an intensive investment into sexual reproduction could spread under a selection pressure, even at the expense somatic maitenance. In contrast, the strategy of asexual individuals can be based upon that the production of offspings with low reproductive value has a low cost as well. In the light of this, the trade-off between reproduction and survival prospectively highlighted in sexual individuals, and can be a major cause of the ageing and somatic maintenance patterns in brown hydras. A possible physiological explanation for the described phenomenon might be found in the availability of limited stem cell resources.

Multipotent stem cells contribute to several life functions in animals with high tissue plasticity, hence intense investment into one function can lead to reduction in other functions, due to the depletion of the stem cell pool. This phenomenon is not a thoroughly studied area, but we know from previous research that the depletion of stem cells is observed in *Aurelia* polyps following subsequent sexual reproduction (Gold & Jacobs, 2013), and the reduced availability of stem cells is responsible for lifespan determination in *Tubularia* cnidarians (Tardent, 1963). The exhaustion of stem cells (gametic crisis; Bosch, 2009; Brien, 1966) is likely responsible for the reduced survival after sexual reproduction in brown hydras as well (Yoshida et al., 2006). During higher reproductive investment, the production of gametes at high numbers requires a commitment of stem cells into the germline pathway at a high proportion, which due to the limitation of stem cells, can reduce the number of cells involved in somatic maintenance and the stem cell pool itself. This is supported by the results of our cell number measurements, because we found a negative correlation between the number of reproductive cells and stem cells together with stinging cells in males. Interestingly, we did not observe such a correlation in females. On the one hand, it could be a result of the fact that the trade-off between germline and somatic cells is different among males and females. On the other hand, it could be because reproductive investment is estimated by gametes in a different way in sexes. In the case of females, many nurse cells gradually become one mature egg during the maturation process (Miller et al., 2000; Zihler, 1972), decreasing the actual number of nurse cells, therefore the reduction of stem cells is accompanied with a reduction of nurse cells as well. This assumption is in accordance with our study, because we found a relatively high number of females with both a low stem cell, stinging cell and nurse cell pool.

In parallel with the stem cell pool, regeneration ability was also decreased in sexually reproducing individuals, while it was maintained in asexuals. The changes in stem cells and regeneration ability can be strongly related, since the role of stem cells is critical in the formation of new body parts, either by their proliferation or movement to the wound (Alvarado, 2000; Bely & Nyberg, 2010; Sugimoto et al., 2011). However, if any physiological process decreases the availability of stem cells, regeneration is also prospectively restricted (Henry & Hart, 2005; Kramarsky-Winter & Loya, 2000). As previous researches support as well, regeneration is part of the allocation strategy and trade-offs (Gross, 1925; Rinkevich & Loyla, 1989; Zattara & Bely, 2013), and the role of available cellular resources is a possible explanation for the restrained regeneration of hydras following subsequent regeneration events (Martinez, 1996; Tardent, 1963). Our investigation confirmed this relatedness, and even highlighted that reproductive modes can differently affect regeneration ability, probably because of the different stem cell requirements of these reproduction modes.

Since regeneration is a plastic trait, age can be one of its influential factors. Prioritized life history traits can change with advancing age, which is a particularly complex phenomenon in partially clonal animals, because different reproductive modes (sexual and asexual) can appear during their lifetime. We put this topic under the microscope in our other experimental research (Study 2). Our results showed that life history traits are significantly influenced by age in brown hydras. Via the experimental induction of sexual reproduction, we discovered that age-dependent reproductive strategies are paired with different somatic maintenance rates. This relation is likely an important part of senescent patterns as well. Although previous studies suggests a tendency describing animals capable of asexual reproduction as organisms with negligible or negative senescene (Vaupel et al., 2004), while the self-maintenance of sexually reproducing animals decline with age (Kirkwood &

Rose, 1991), our study shows that these patterns can change during their lifetime.

To understand allocation patterns and priorities of traits affecting major life history components in clonal and non-clonal organisms, such as growth, reproduction, regeneration and self-maintenance, we have to consider a substantial difference between the two groups. Importantly, growth is not just an increase in size in clonal and partially clonal organisms, but it means production of offspring from the body and somatic cells as well (Harvell & Grosberg, 1988). Asexual reproduction enables a genotype adapted to local conditions to quickly colonize a habitat at a low expense (Rispe et al., 1998). Therefore, in contrary with non-clonal organisms, an investment into asexual reproduction can be advantageous early in life, in case of good condition (Burke & Bonduriansky, 2018). In partially clonal organisms, the increase of genetic diversity via sexual reproduction might become beneficial only later in life (Burke & Bonduriansky, 2018). Our research supports this hypothesis, since we found that asexual reproduction is more important early in life in brown hydras, while sexual reproduction is priotized later. These results confirm former studies, in which scientists described fewer eggs at young age in female stick insects (Burke & Bonduriansky, 2018), age-dependent sexual maturation in some coral species (Kai & Sakai, 2008) and preceeding asexual cycles before sexual reproduction in some partially clonal species (Olive, 2002). Although there are examples for these age-dependent changes, the reproductive patterns and experimental induction of change between reproductive modes is much less studied, albeit these are indispensable to evaluate the influential factors and costs and benefits of reproductive mode switch.

In our experiment, reproductive modes were associated with different somatic costs: in younger individuals higher asexual reproduction rate and delayed, less intense gematogenesis was paired with higher regenration and survival, while the opposite was true for older polyps. The higher regeneration ability and survival associated with asexual reproduction has been shown in other partially clonal species (Mouton et al., 2018; Zattara & Bely, 2016), and the negative effect of sexual reproduction on these is also known (Harvell & Grosberg, 1988; Henry & Hart, 2005). Regarding to age-dependent changes, a general tendency shows that asexual reproduction is usually not associated with declining regeneration ability and survival (Tanner, 2001; Yun, 2015), however, a decrease in these traits occurs in some cases (Meesters & Bak, 1995; Orive, 1995; Sköld et al., 2011). This inconsistency might be explained by the dependence of age-dependent changes on self-maintenance on other life history traits (Orive, 1995), such as reproductive modes presented in our experiment. However, former studies usually did not investigate the two reproductive modes in parallel with self-maintenance changes, and did not consider age-dependent changes, which is highlighted by our results.

At a proximal level, our cellular measurements likely explain the increasing propensity for sexual reproduction with age and its higher cost. Gametes are produced by germline stem cells in hydras, which cells originate from multipotent stem cells responsible for also somatic cells (stinging, gland and nerve cells) production (Nishimiya-Fujisawa & Kobayashi, 2012). For this reason, the differentiation of multipotent stem cells can shift to the gamete production pathway due to maturation, instead of somatic cell production. The reduced availability of stem cells for somatic functions might indicate the main cost of sexual reproduction, since somatic cells are responsible for regeneration, asexual reproduction and the maintenance of the body in general (Nishimiya-Fujisawa & Kobayashi, 2012). Increasing number of germline stem cells with age has been documented in female hydras (Littlefield, 1991), showing a preparedness for sexual reproduction. In light of this, it is possible that younger animals in our experiment were not able to produce gametes at a high number, because they did not have an adequate amount of germline stem cells. The temporal dynamics of gametes and stem cells, together with regeneration measurements, suggest an ontegentic effect as a possible further explanation to our results. In these measurements, younger animals belonging to the '1 week' group showed a 3 week shift compared to the '4 week' group. At the same time, when we controlled for absolute age (age calculated from the detachment of parent polyps) of animals, the effect of age on sexual reproduction still remained significant. Hence overall, an ontogenetic effect and age-dependent plasticity likely together played a role in the differences of life history strategies in brown hydras.

As age and senescence progresses, there is a decreasing tendency in physiological regeneration (Yun, 2015), which is a key for survival. The effect of age is considered by ageing theories, but the availability of resources receives little scientific interest (Baudisch & Vaupel, 2012). According to the allocation theory, food is one of the fundamental resources for the expression and function of life history traits, and it is probably in relation to the interesting life history strategy of hydras. We investigated this in our third experiment (Study 3), in which we measured sexual reproduction and following survival at 4 food levels in 10 asexually propagated strains of the species *Hydra oligactis* with post-reproductive senescence.

In our experiment, the number of sexual organs increased with amount of food, however,the initiation of gonadogenesis and survival after sexual reproduction were independent from food levels. Based on our results, it seems that - at the used food levels - brown hydras are able to preserve survival ability, even in case of sexual reproduction. These results are surprising in many aspects. Firstly, sexual reproduction is thought to be a type of escaping strategy in brown hydras, and the production of resting eggs is required because of frost and/or starvation causing high mortality. Our observation does not confirm this hypothesis, since the amount of food did not have an effect on the probability of sexual reproduction initiation (similar to Kaliszewicz & Lipińska (2012)). The hypothesis above is also not confirmed by the fact that asexual reproduction was not constantly impeded at low temperature, and animals were able to produce asexual offsprings (namely buds) before the initiation of sexual reproduction. This suggests that temperature drop is rather cue to ecologically optimal sexual reproduction, than a physiological constraint (Reisa, 1973).

Furthermore, it is also surprising that although we observed the degeneration, body and tentacle size shrinkage and death after sexual reproduction as previously reported (Yoshida et al., 2006), a substantial proportion of animals were able to regenerate, survive and reproduce asexually. This pattern is not explainable by either the phenomenon of terminal investment or dietary restriction, or by a model in which both reproduction and survival are dependent on food availability. Like I mentioned related to the previous experiments (Study 1 and 2), the main cause of intensive post-reproductive degeneration is probably the exhaustion of stem cells (Bosch, 2008; Brien, 1953; Reisa, 1973; Tardent, 1974; Yoshida et al., 2006). Based on the theory of “gametic crisis”, a plausible explanation for the high post-reproductive survival rate is that in these strains, stem cells were likely committed to the germline pathway at a lower rate. Therefore, a sufficiently big population of stem cells remained, and was able to regenerate the whole body. Considering that the minimum tissue size required for whole body regeneration is really small in hydras (Shimizu et al., 1993), this is a potential explanation.

Thirdly, our results do not support the hypothesis, according to which post-reproductive degeneration is a consequence of reduced allocation of food resources to somatic functions. Food availability did not have an effect on survival rate, and we did not find a relation between the number of sexual organs and survival at an individual level, what would be a key component of the hypothesis relying on trade-offs. However, it is possible that the number strains involved in our experiment was not enough to detect such a relation.

Nevertheless, the 10 strains involved in our investigation highlight the possible presence of different life history strategies within the species. Even within this relatively restricted number of strains, there were strains consisting of clones, which reproduced sexually at a high rate and which remained asexuals during the measurements (results not shown). Furthermore, the survival probability of some strains was really high, while in others it was lower than 25 %. These differences can be a part of different life history strategies, or maybe caused by a periodically changing selection pressure on life history traits (Mojica et al., 2012). The high survival rate following sexual reproduction observed in many strains, is rather a characteristic of iteroparous animals. In these animals the maintenance of somatic functions is often documented, even via the reduction of reproduction rate in challenging circumstances like in terms of food (e.g., Gaillard et al., 2000; Therrien et al., 2008). On that measure, the relation of food availability, survival and reproduction can differ between iteroparous strains/population and semelparous ones with low survival. Although these two mortality patterns are shown by a few former studies (Tomczyk et al., 2015; Yoshida et al., 2006), to test the hypothesis above, we need to investigate varying strains and populations at larger scale in the future.

Taking everything into account, in my dissertation work we could discover new relations in several aspects of regeneration, which may facilitate new research directions. Investigating sexual and asexual reproductive modes, stem cell pool and head regeneration of brown hydras, we found that sexual reproduction has higher physiological costs (Study 1). Based on the reduced regeneration ability and depletion of stem cells, sexual reproduction seems to be an irreversible reproduction strategy, prioritizing gamete production at the expense of somatic functions. The divergent, reproductive mode dependent life history decisions make hydras an ideal model organism for investigating senescent and non-senescent strategies, even in terms of physiology. In order to explicitly clarify the role of limited cellular resources, there is a much need for further studies investigating common cell pools required by several life history traits.

The patterns of sexual and asexual reproduction together with stem cells were also significant in our later experiments, but we extended these with a new factor, and has begun the discovery of age-dependent changes (Study 2). We performed an experiment with one hydra strain, and our results suggests that asexual reproduction together with regeneration and self-maintenance is prioritized at younger age, while sexual reproduction with its higher somatic costs is only important later, if sex is induced. The significant age-dependent plasticity in brown hydra clones can highlight a new, developed direction of research done with clonal or partially clonal species kept in the laboratory. Various types of asexual reproduction is widespread among model organisms (for example planarians, water fleas, rotatorias, sea anemones or hydras), which are kept in asexually propagated strain cultures containing animals with different age (Hughes, 1989 and references therein). Our study implies that the same environmental effects can induce different reproductive and resource allocation patterns in brown hydra polyps, depending on their age. Accordingly, this phenomenon can be present in other clonal or partially clonal organisms, therefore controlling for age might help to enhance repeatability in other scientific fields as well.

Lastly, related to physiological regeneration, we aimed to shed light on the background of drastic reduction of survival after sexual reproduction in brown hydras, via investigating food availability (Study 3). Since the amount of available food had an effect only on the number of sexual organs but not on later survival, it is unclear whether the inhibition of somatic function maintenance is a result of reduced resource allocation. However surprisingly, a notable proportion of animals were able to regenerate from degeneration after sexual reproduction, therefore raised the possibility of a subsequent, iteroparous strategy in this species. Different strains show marked variance both in the inducibility of sexual reproduction and survival after sexual reproduction, which differences necessitate extensive population research.

Overall, my dissertation work suggests that the investigation of regeneration, as an important life history trait can help to understand life history trade-offs, differing senescent patterns and their causes, and the physiological background of these. Via our research, we have demonstrated that brown hydras are suitable for all of these study purposes.

2.6. Summary

Regeneration is a fundamental feature of living organisms, and via restoration and rejuvenation processes, enables the resistance of cells and tissues against damages and injuries. It has a widespread role, participating in developmental pattern formation, asexual reproduction and senescence as well. Life history traits, therefore regenerative processes require several costly mechanisms, hence trade-off networks inevitably occur between life history components. Regeneration is involved in trade-offs among contrary life history components in many organisms, such as reproduction and self-maintenance, however, its relation to other traits responsible for self-maintenance is not well known. Studying brown hydras (*Hydra oligactis*), I investigated the nexus of regeneration and other traits responsible for self-maintenance and reproduction, depending on age and food availability. On the other hand, in my dissertation work I also studied the consequences of competition for internal, limited resources, as a determinant factor for trade-offs. In animals with high tissue plasticity, therefore in freshwater hydras as well, stem cells are potentially such a determinant factor, since both their somatic and reproductive cells originate from multi- or

pluripotent stem cells. Due to this physiological background, the competition for limited stem resources in case of sexual reproduction can affect regeneration as well.

Our results confirmed the mediatory role of stem cells in shaping somatic and reproductive functions. In terms of reproductive modes, we found that sexual reproduction has higher costs than asexual, both as stem cell resources and regenration consequences. However, the rate of engagement to seemingly irreversible sexual reproduction can be dependent on individual age. Asexual reproduction and regeneration is prioritized in younger animals, while sexual reproduction with higher somatic costs is more important later in life. The age-dependent life history changes in the same environment can be true for other clonal or partially clonal animals as well, hence controlling for age can facilitate increasing repeatability in other scientific fields.

We also investigated food availability, as a factor affecting physiological (maintenance) regeneration, including several brown hydra strains. Although the amount of food had a significant effect on the number of sexual organs, it did not affect survival, thus further research is required to clear the role of food availability. At the same time, we found marked differences in survival between hydra strains, because surprisingly, a large proportion of animals was able to regenerate form degeneration following sexual reproduction, while another part of the animals died - as expected based on former studies. These notable differences suggest the necessity of an extensive population study.

Overall, my dissertation work suggests that the investigation of regeneration, as an important life history trait can help to understand trade-off networks, differing senescent patterns and their causes, and the physiological background of these. Via our research, we have demonstrated that brown hydras are suitable for all these study purposes.

2.7. Publications related to the thesis

I Sebestyén, F., Barta, Z., & Tökölyi, J. (2018). Reproductive mode, stem cells and regeneration in a freshwater cnidarian with postreproductive senescence. Functional Ecology, 32(11), 2497-2508.

II Sebestyén, F., Miklós, M., Iván, K., & Tökölyi, J. (2020). Age-dependent plasticity in reproductive investment, regeneration capacity and survival in a partially clonal animal (*Hydra oligactis*). Journal of Animal Ecology, 89(10), 2246-2257.

III Tökölyi, J., Ősz, Z., Sebestyén, F., & Barta, Z. (2017). Resource allocation and post-reproductive degeneration in the freshwater cnidarian *Hydra oligactis* (Pallas, 1766). Zoology, 120, 110-116.

2.7.1. Personal contribution (*) to the publications related to the thesis

	Study 1	Study 2	Study 3
Research conception	*	*	
Data collection	*	*	*
Data analysis	*	*	
Manuscript preparation	*	*	*

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4. Függelék / Supplement

4.1. 1. tanulmány / Study 1: Reproductive mode, stem cells and regeneration in a freshwater cnidarian with postreproductive senescence

Reproductive mode, stem cells and regeneration in a freshwater cnidarian with post-reproductive senescence

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Abstract

1. In many basal metazoans both somatic and reproductive functions are performed by cellular derivatives of a single multipotent stem cell population. Reproduction can drain these stem cell pools, imposing a physiological cost with subsequent negative effects on somatic maintenance functions.

2. In the freshwater cnidarian *Hydra oligactis* both asexual (budding) and sexual reproductive modes (production of resting eggs) are present, and both of these are dependent on a common pool of interstitial stem cells. Resting eggs tolerate harsh abiotic conditions which neither the parental animals, nor asexual offspring can survive (e.g. freezing). Therefore, when facing unfavourable conditions and increased mortality risk, hydra polyps are expected to show higher level of differentiation of interstitial stem cells into germ cells (i.e. sexual reproduction) than other cell types needed for self-maintenance or asexual reproduction.

3. Here, by comparing sexually and asexually reproducing individuals to non-reproductives, we studied the physiological costs of reproduction (size of interstitial stem cell pools, their somatic derivatives and regeneration rate, which is dependent on these cell types) in *H. oligactis* polyps from a free-living Hungarian population prior to the onset of winter.

4. Sexual individuals were characterized by significantly smaller interstitial stem cell pools, fewer nematoblasts involved in food capture and lower regeneration ability compared to non-reproductives, but asexuals did not differ from non-reproductive animals. We also found a negative correlation between germ cell counts and stem cell numbers in males (but not in females).

5. We suggest that the lower numbers of these cell types and lower regenerative ability in sexual individuals reflect a somatic cost of sexual reproduction. Our results also suggest that increased differentiation of stem cells into gametes might limit investment into somatic functions in hydra polyps. Exhaustion of cellular resources (stem cells) could be a major mechanism behind the extreme post-reproductive senescence observed in this species.

Keywords: cost of reproduction, Eco-Evo-Devo, Hydra, interstitial cells, life history trade-offs, semelparity.

Introduction

Sexual reproduction is ubiquitous in the natural world. Sex has clear evolutionary benefits over asexual reproduction (such as producing recombinant genotypes that have higher fitness under changing conditions), but it also entails costs (such as the cost of producing males; Maynard Smith, 1978). Asexual reproduction does not entail these costs and can be evolutionarily favoured under special conditions (Crow, 1994). Both sexual and asexual reproduction have a common cost: that of investing resources into offspring at the expense of self-maintenance of the parent (the physiological cost of reproduction; Calow, 1979; Harshman & Zera, 2007; Flatt & Heyland, 2011). In animals, commonly studied physiological costs of reproduction are the drain of specific macronutrients (e.g. amino acids, proteins or carbohydrates; Zera & Zhao, 2006; Cotter, Simpson, Raubenheimer & Wilson, 2011), micronutrients (e.g. dietary antioxidants; Catoni, Peters & Schaefer, 2008) or metabolic reserves (e.g. body fat; Ellers, 1995). However, any factor in limited supply that is required by multiple life functions can mediate trade-offs between reproduction and somatic maintenance.

In animals with high tissue plasticity – like sponges, cnidarians and flatworms – stem cells might represent such a limiting factor. While in adult vertebrates stem cells have only limited plasticity (Weissman, 2000), in some invertebrates the adult body contains populations of highly flexible multi- or pluripotent stem cells (e.g. archeocytes in sponges, interstitial cells in some cnidarians, neoblasts in flatworms) which are responsible for the maintenance of a wide range of functions through their derivatives (Extavour & Akam, 2003; Juliano, Swartz & Wessel, 2010; Gold & Jacobs, 2013; Kumano, 2015). The important role of these stem cells in self-maintenance is clearly seen in hydrozoans where, for instance, interstitial cells give rise to nerve cells, nematocytes (stinging cells usable once to capture food) and gland cells involved in digestion (Bode, 1996; Bosch, 2009; David, 2012; Plickert, Frank & Müller, 2012). The availability of these cells (i.e. cellular resources) is thought to determine growth rate and the magnitude of regenerative processes in sponges, corals and hydrozoans (Tardent, 1963; Lang da Silveira & Van't Hof, 1977; Simpson, 1984;

Rinkevich, 1996; Henry & Hart, 2005) and could be of key importance in the extreme longevity of hydra polyps (Bosch, 2008; Boehm et al., 2013; Schaible et al., 2014; Schaible et al., 2015). Experimental elimination of stem cells in the freshwater cnidarian *Hydra* impairs several life functions related to the descendant cell types (Diehl & Burnett, 1964; Marcum & Campbell, 1978; Sugiyama & Wanek, 1993). Multipotent stem cells are also strongly involved in reproduction: they are incorporated into asexual offspring during fission, fragmentation or budding (in sponges: Simpson, 1984; in hydrozoans: Bode, 1996; Gahan, Bradshaw, Flici & Frank, 2016), produce resting bodies (gemmules and reduction bodies in sponges; Simpson, 1984), and give rise to germ cells (in sponges: Simpson, 1984; in hydrozoans: Bosch & David 1987; Gahan, Bradshaw, Flici & Frank, 2016; in planarians: Newmark, Wang & Chong, 2008) or germline stem cells (a less potent stem cell lineage which can differentiate into gametes but not somatic cells in *Hydra*; Nishimiya-Fujisawa & Kobayashi, 2012).

The common involvement of a single pool of multipotent progenitors in both somatic and reproductive functions, in theory, implies that increased investment into reproduction (either sexual or asexual) will result in higher physiological cost of reproduction either through reducing differentiation of stem cells into somatic derivatives (Rinkevich, 1996; Henry & Hart 2005) or by increasing metabolic expenditure required for an accelerated cell cycle producing germline and somatic cells. However, the mode of reproduction can be different and if the expected reproductive value of sexual and asexual offspring is not equal, then reproductive investment into these offspring types (and costs on stem cells) should also differ. Such a difference in reproductive value between offspring types could arise e.g. if expected survival rate of sexual and asexual offspring is not identical. Indeed, differential investment into sexual and asexual reproduction is commonly seen in several animal groups (e.g. ascidians: Yund, Marcum & Stewart-Savage, 1997; aphids: Nespolo, Halkett & Figueroa, 2009; *Daphnia*: Innes & Singleton 2000; freshwater hydra: Kaliszewicz, 2011). However, much less is known about the physiological consequences of this differential allocation.

The freshwater cnidarian *Hydra oligactis* is a species with a mostly temperate/arctic distribution in the Northern Hemisphere (Martínez et al., 2010). *H. oligactis* polyps reproduce asexually throughout the year, but switch to sexual reproduction during the autumn (Schuchert, 2010). The most commonly invoked explanation for this switch in reproductive mode is to produce resting eggs that can survive the winter (Reisa, 1973). Based on laboratory experiments, sexual reproduction is followed by a senescence-like degeneration and increased mortality of polyps (Brien, 1953; Yoshida, Fujisawa, Hwang, Ikeo & Gojobori, 2006; Tomczyk, Fischer, Austad & Galliot, 2015; Tökölyi, Ősz, Sebestyén & Barta, 2017; Schenkelaars et al., 2017; Tomczyk et al., 2017). Post-reproductive degeneration is accompanied by marked changes in the cellular composition: interstitital cell populations are strongly reduced while reproductive cells increase in number (Tardent, 1974; Yoshida, Fujisawa, Hwang, Ikeo & Gojobori, 2006). Because of these changes, post-reproductive senescence in hydra is hypothesized to be the consequence of "gametic crisis", in which stem cell populations become exhausted due to excessive differentiation into reproductive cells, limiting their involvement in somatic functions (Brien, 1966; Tardent, 1968; Tardent, 1974; Bosch, 2009). All these observations were done on laboratory strains and the role of cellular resources in mediating the trade-off between reproduction and self-maintenance is poorly understood in natural populations of hydra (or any other taxon). To date, the role of the stem cell pool in this trade-off is mostly suspected on the basis of the negative linkage, reported only in a handful of cases, between (i) traits depending on stem cells (like suppressed regeneration during reproduction (Campbell, 1967)), and (ii) the actual depletion of stem cells after initiation of sexual reproduction (Littlefield, 1985; Yoshida, Fujisawa, Hwang, Ikeo & Gojobori, 2006; Gold & Jacobs, 2013). Post-reproductive senescence and stem-cell depletion has been described in *H. oligactis* (Yoshida, Fujisawa, Hwang, Ikeo & Gojobori, 2006), but previous studies worked only with a few laboratory strains and it is unclear whether variation in reproductive strategies between strains are associated with patterns of stem cell loss and changes in regeneration ability, as would be predicted from life history theory.

In this study, we investigated cellular composition and regeneration rate in *H. oligactis* polyps differing in reproductive modes (sexually, asexually reproducing and non-reproductive individuals), sampled during the autumn sexual period from their natural environment. We hypothesized that the reproductive value of sexual offspring should be higher close to the end of autumn because eggs produced through sexual reproduction can survive freezing of water, while asexual offspring cannot. As a consequence, sexual individuals should invest more into reproduction, which would result in higher overall physiological cost of reproduction. Supporting the mediator role of the stem cell pool, this would manifest itself in lower availability of stem cells, and hence their somatic derivatives and somatic functions depending on stem cells as well. Accordingly, we predicted lower number of stem cells, fewer nematoblasts (indicating reduced differentiation into somatic functions) and lower ability to regenerate in sexual individuals compared to asexuals or non-reproductives. Furthermore, we also predicted that, if differentiation of stem cells into reproductive function is traded off with somatic maintenance, then the number of reproductive cells should be negatively related to interstitial stem cells and their somatic derivatives.

Materials & methods

Collection of animals and culture conditions

Experimental animals were collected from a shallow (average depth ~1m) oxbow lake near Tiszadorogma in Eastern Hungary (47.6712N, 20.8641E; Tökölyi, Ősz, Sebestyén & Barta, 2017). To determine the reproductive status of the animals in the lake and hence to detect the start of the autumn reproductive period we visited the site on 2nd and 16th October 2016 and collected N = 168 (N = Number of collected animals) and N = 136 hydra polyps, respectively. Further collections were performed four times later in 2016: 26th October (N = 127), 2nd November (N = 332), 15th November (N = 121) and 6th December (N = 51, in total 631 individuals). Animals collected on the first two dates were not used in regeneration experiments or for quantification of cellular composition, only for the detection of sexual reproduction period. We collected animals from several localities along the shoreline of the lake to reduce the chance of obtaining genetically identical clones produced by asexual budding. Hydras were picked up from submerged vegetation, placed in Eppendorf tubes and brought to the laboratory in a cool box on the same day. In the laboratory, we recorded the mode of reproduction according to three categories: (1) no reproduction (polyps without buds or gonads, N=272), (2) asexual reproduction (polyps with at least one bud, N=204), or (3) sexual reproduction (polyps with differentiated or developing gonads; this latter was defined as a thick, opaque swelling around the gastric region of the body column, N=155). Sexual individuals were further divided into three categories: males (polyps with differentiated testes, N=25), females (polyps with differentiated eggs, N=34), and sexual individuals in which sex could not be determined (N=96). This latter category included immature males and females with developing testes or eggs, and post-reproductives showing the morphological characteristics of sexual reproduction, but without clearly defined reproductive organs, since these categories are not unambiguously distinguishable. Animals were clearly referable to only one category of reproduction modes (we found just two asexual animals showing the morphological signs of sexual reproduction; these were coded as sexual individuals because gonadogenesis was clearly initiated).

Head regeneration measurements

About half of the collected animals (altogether 338) were randomly assigned to head regeneration measurements, which were initiated one day after each of the four collections. Animals were decapitated below the tentacles, which means that the removed part contained the oral tip (i.e. the hypostome), the tentacles and a short part of the trunk (~10% of the body length). During regeneration we kept the animals individually in 24-well plates in ~ 3 ml standard hydra medium (1.0 mM CaCl2, 0.1 mM MgCl2, 0.03 mM KNO3, 0.5 mM NaHCO3, 0.08 mM MgSO4; Zhang et al., 2002). We placed the plates with hydras in a Memmert ICP 700 climate chamber and kept them on constant photoperiod (16 h dark/ 8 h light cycle) and temperature in accordance with natural habitat temperature measurements on the four consecutive dates (12 ºC, 9 ºC, 5 ºC, 4 ºC, measured approximately 20 cm below the water surface on the day of collection). Hydras completely regenerate their head after 48-72 h on 18 ºC (Ambrosone et al., 2012), but at lower temperature cell cycle and cell division is slower (Begasse, Leaver, Vazquez, Grill & Hyman, 2015), thus regeneration takes longer (Lillie & Knowlton, 1897). For this reason, we recorded regeneration 4 days after decapitation by a binary code system, based on the presence or absence of newly emerged tentacles.

The hypostome and tentacles amputated for the head regeneration experiments were used for species determination. *H. oligactis* can be distinguished from other *Hydra* species occurring at this site based on nematocyte morphology, which can be observed under a light microscope.

Cell number measurement

One day after each collection, we randomly selected a subset (altogether 155 animals) from the remainder of the animals for cell number measurement. These were macerated according to the standard procedure described by David (1973), and then cells were spread on a microscopic slide. Sample size for the cell number measurement was determined by time constraints: we only used samples for which macerations could be prepared on the next day after collection, such that cellular composition measured by us is as close as possible to the condition of animals at the time of sampling. Cellular composition was quantified within a few days after maceration. For each sample we recorded the number of epithelial cells, interstitial stem cells (large single interstitial cells or nests of two interstitial cells were recorded together to obtain an estimate of the frequency of stem cells; Bosch & David, 1987), nematoblast nests (total number of nests of 1, 2, 3-4, 5-8 or >8 cells) and reproductive cells (sperm precursor nests in males and nurse cells in females). Reproductive cells at later stages of development are distinguishable from interstitial cells based on morphological criteria, as follows. In males, interstitial cell nests committed to sperm development increase in number and size and flagella start to develop on the sperm precursors (Littlefield, 1985). We used the presence of flagella as a morphological criterion to identify sperm cells/sperm precursors and counted the number of sperm precursor nests (i.e. groups of sperm precursors with flagella) to obtain an estimate of minimal germ cell numbers in males (the actual number can be higher than this estimate because sperm precursor nests can contain more than one germ cell but the large number of individual germ cells in some nests made exact counting of cell numbers impossible). In females, interstitial cells committed to germ cell differentiation first increase in size and develop into nurse cells, which can be distinguished from interstitial cells by their larger cytoplasm volume (Zihler, 1972). Cells were identified as nurse cells when the diameter of the nucleus was equal to or less than half of the cell diameter, indicating relatively large cytoplasm volume. This corresponds to Stage B oogonia in Zihler's (1972) notation. Only a small subset of these nurse cells develop into oocytes, but these incorporate neighbouring nurse cells through phagocytosis (Miller, Technau, Smith & Steele, 2000). Hence all nurse cells contribute to reproduction and therefore we counted all of them.

In all samples we systematically traversed slides until at least one hundred epithelial cells were recorded, and noted any other cell types alongside these epithelial cells. The median number of cells/cell nests recorded per sample (including the epithelial cells) was 208 (range: 107-3048).

The head region of the animals assigned to investigation of cellular composition was removed in the same way as in the head regeneration experiments and used for species determination. All sexual individuals involved in cell number measurements were categorized as males or females, based on the presence of mature gonads and / or sperm cells / nurse cells in macerates.

Statistical analysis

The effect of reproductive mode on head regeneration was analysed using Generalized Linear Mixed Models (GLMM) with binomial distribution. Our model contained regeneration (presence or absence) as dependent variable and reproduction mode as predictor. We included collection date as a fixed effect, to control for seasonal and temperature differences. We included collection site as a random effect to control for the possibility that animals from the same sampling point might be more similar to each other than to individuals from other sites because of shared environment or because some of them might be asexual descendants of a single individual. Binomial GLMMs were implemented in a Bayesian framework, employing the MCMCglmm R package (Hadfield, 2010; R Core Team, 2017). A Bayesian approach was required because our data suffered from complete separation (some experimental groups contained only non-regenerating animals). This problem can be circumvented in a Bayesian model by setting a weak prior on fixed effects in MCMCglmm. We ran this model two times for our data sets then averaged the two results.

For testing the effect of reproductive mode on nematoblast and interstitial cell number, we used Poisson GLMM also implemented in a Bayesian framework. We used either none-reproductive animals or asexuals as reference levels in the reproductive mode to test the difference between all three groups (see below). We included epithelial cell number as a fixed effect, because the number of epithelial cells was not exactly identical (sometimes we counted slightly more than one hundred); by controlling for epithelial cell number we take into account variation in stem cell numbers arising from slightly unequal sampling. We also included collection date as a fixed effect and collection site as a random effect for the reasons mentioned above.

For testing the effect of reproduction modes on head regeneration, nematoblast and interstitial cell number first we compared each reproductive mode category to non-reproductive animals, then we used asexuals as a reference level to perform comparison between sexual (males, females and sex undetermined) and asexual categories as well. For analyzing the relation between sperm/nurse cell number and interstitial or nematoblast cell number we performed Spearman rank correlation. Cell type numbers were normalized to epithelial cell number. All analyses were performed in the R Statistical Environment version 3.4.4 (R Core Team, 2017).

Results

Reproductive phenology

None of the individuals collected on 2nd October showed signs of sexual development. A single male bearing mature testes was observed on 16th October. The proportion of sexual individuals on subsequent dates was 20.5%, 29.8%, 22.3% and 5.9% on 26th October, 2nd November, 15th November and 6th December, respectively (Fig. 1). The proportion of asexual animals was 18.1%, 30.1%, 46.3% and 49%, on the respective dates (Fig. 1).

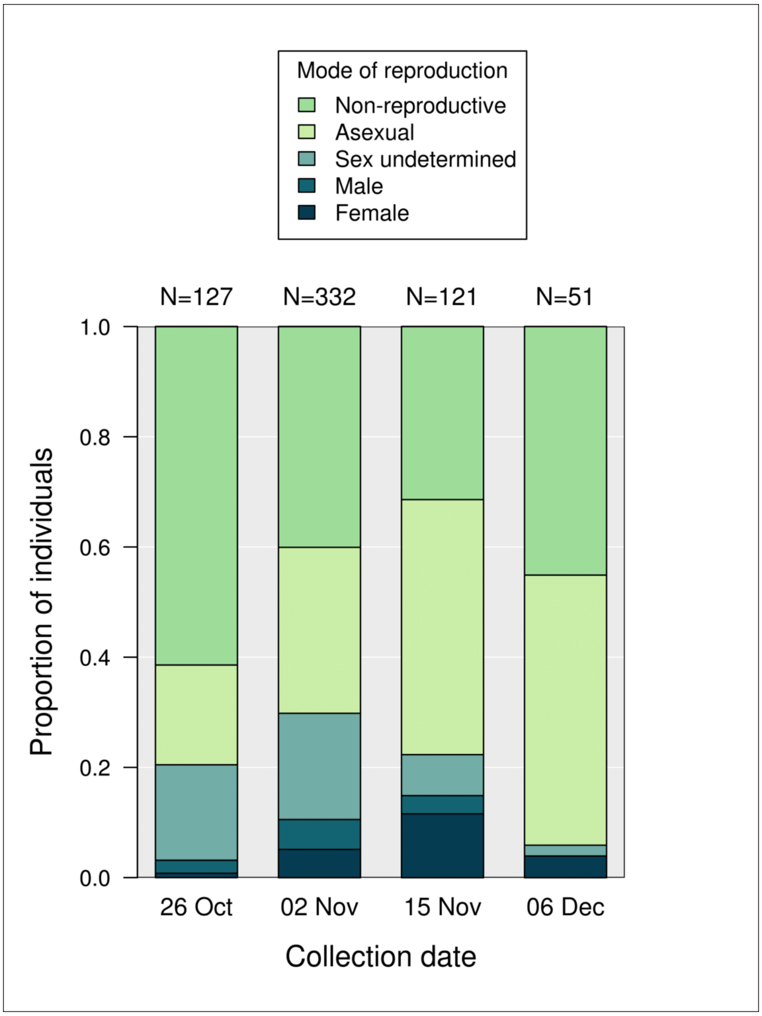

Fig. 1. Proportion of individuals in different reproduction mode categories on four collection dates. Total sample sizes are shown above the bars. Reproductive mode categories were as follows: nonreproductive (polyps without buds or gonads), asexual (polyps with at least one bud) and sexual (polyps with differentiated eggs [females], testes [males] or developing gonads [sex undetermined]).

Head regeneration

Regeneration abilities differed between reproduction mode categories (Fig. 2). Compared to non-reproductive animals (56.85% regenerated heads within 4 days based on recorded data), the proportion of animals regenerating heads was significantly lower in males (0%; posterior mean = -5.431, lower 95% CI = -8.855, upper 95% CI = -2.157, p<0.001), females (0%; posterior mean = -4.667, lower 95% CI = -8.545, upper 95% CI = -1.542, p<0.001) and animals with undetermined sex (23.21%; posterior mean= -2.341, lower 95% CI= -3.353, upper 95% CI= -1.351, p<0.001). Compared to asexual individuals, sexually reproducing animals had significantly lower head regeneration rate: males (posterior mean = -4.880 , lower 95% CI = -8.650, upper 95% CI = -1.824 , p<0.001) females (posterior mean =-4.110, lower 95% CI = -7.954, upper 95% CI = -0.797 , p=0.003) and animals with undetermined sex (posterior mean = -1.659, lower 95% CI = -2.788, upper 95% CI = -0.544, p=0.004) as well. In asexual hydras, head regeneration did not differ significantly from non-reproductive individuals (30.61%; posterior mean = 0.568, lower 95% CI = -1.447, upper 95% CI = 0.252, p = 0.179). Collection date as a fix effect significantly affected regeneration rate: compared to the first collection, regeneration rate was significantly lower in all dates (results not shown).

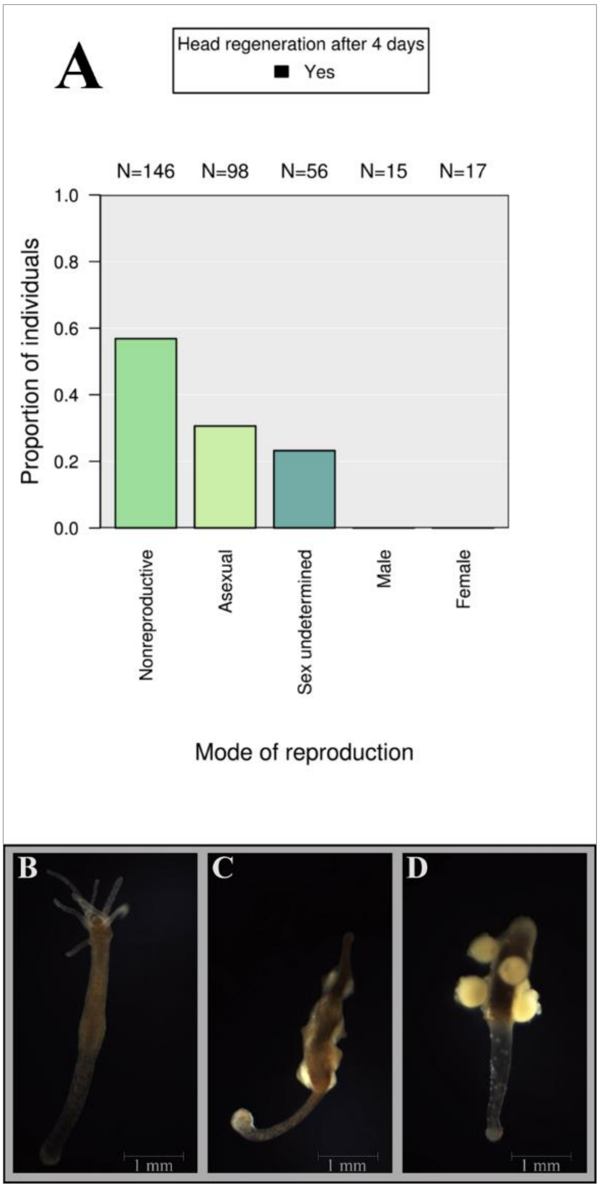

Fig. 2. Head regeneration (presence or absence of tentacles) 4 days after decapitation in hydras differing in reproductive mode (a). Nonreproductive (b), male (c) and female (d) polyp after decapitation illustrating the markedly reduced head regeneration ability of sexual individuals. See Figure 1. For reproductive mode categories. Photographs were taken after finalization of regeneration experiments (8 days postdecapitation).

Nematoblast and interstitial cell number and mode of reproduction

Mode of reproduction had a significant effect on both nematoblast and interstitial cell number, after controlling for the number of epithelials (Fig. 3). Compared to non-reproductive animals, interstitial cell number did not differ in asexually reproducing individuals (posterior mean = 0.183, lower 95% CI = -0.320, upper 95% CI = 0.735, p = 0.519), but it was lower in males (posterior mean= -0.875, lower 95% CI = -1.564, upper 95% CI = -0.212, p=0.009) and females (posterior mean = -1.197, lower 95% CI = -1.672, upper 95% CI = -0.742, p < 0.001). There was a significant difference between asexual and sexual animals: both males (posterior mean = -1.048, lower 95% CI = -1.728, upper 95% CI = -0.352, p<0.001) and females (posterior mean = -1.372, lower 95% CI = -1.888, upper 95% CI = -0.858, p<0.001) had lower interstitial stem number compared to asexuals. Nematoblast cell number was significantly lower in males (posterior mean = -1.196, lower 95% CI = -1.937, upper 95% CI = -0.416, p < 0.001) and females (posterior mean = -1.929, lower 95% CI = -2.488, upper 95% CI = -1.383, p < 0.001), but it was slightly, but non-significantly higher in asexual animals (posterior mean = 0.578, lower 95% CI = -0.023 upper 95% CI = 1.144, p = 0.055), compared to non-reproductives. Compared to asexuals, both males (posterior mean = -1.750, lower 95% CI = -2.531, upper 95% CI = -0.986, p<0.001) and females (posterior mean = -2.470, lower 95% CI = -3.030, upper 95% CI = -1.851, p<0.001) had significantly lower nematoblast cell number.

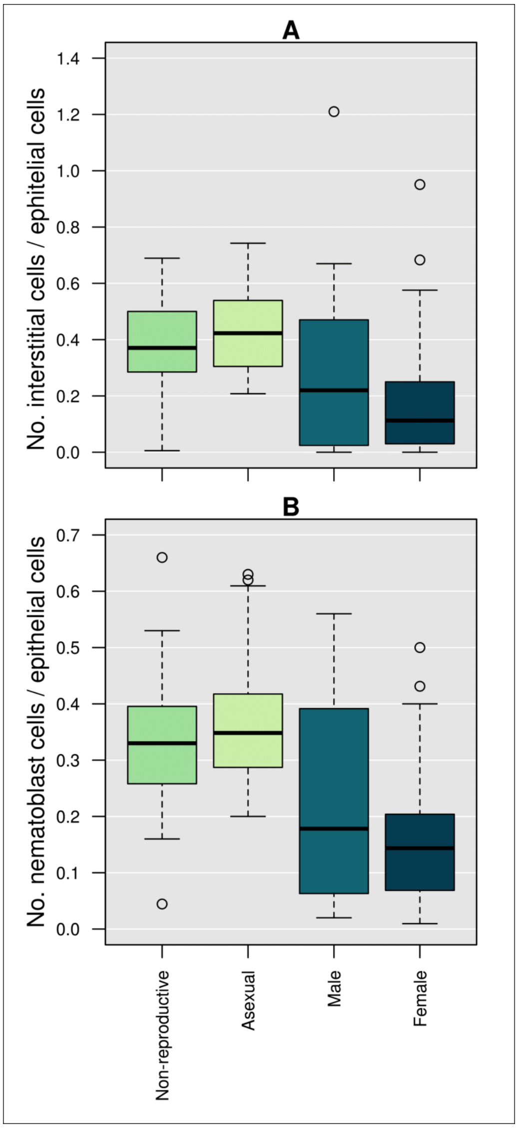

Fig.3. Relative interstitial stem cell number (a) and nematoblast number (b) of individuals in different reproductive mode categories. All sexual individuals were categorized as males or females based on the presence of mature gonads and/or sperm cells/nurse cells in macerates.

Reproductive and interstitial cell number

There was a significant negative correlation between number of sperm precursor nests and interstitial cell number (Spearman correlation, ρ= -0.756, p<0.001, N=22), as well as number of sperm precursor nests and nematoblast cell number in males (Spearman correlation, ρ = -0.878, p<0.001, N=22) (Fig. 4). We found a significant positive correlation between nurse cell number and interstitial cell number in females (Spearman correlation, ρ = 0.444, p=0.001, N=51), but there was no correlation between their nurse cell number and nematoblast number (Spearman correlation, ρ = 0.147, p=0.305, N=51) (Fig. 4). There was a significant positive correlation between nematoblast and interstitial cell counts in both males (Spearman correlation, ρ= 0.558, p=0.010, N=22) and females (Spearman correlation, ρ = 0.516, p<0.001, N=51).

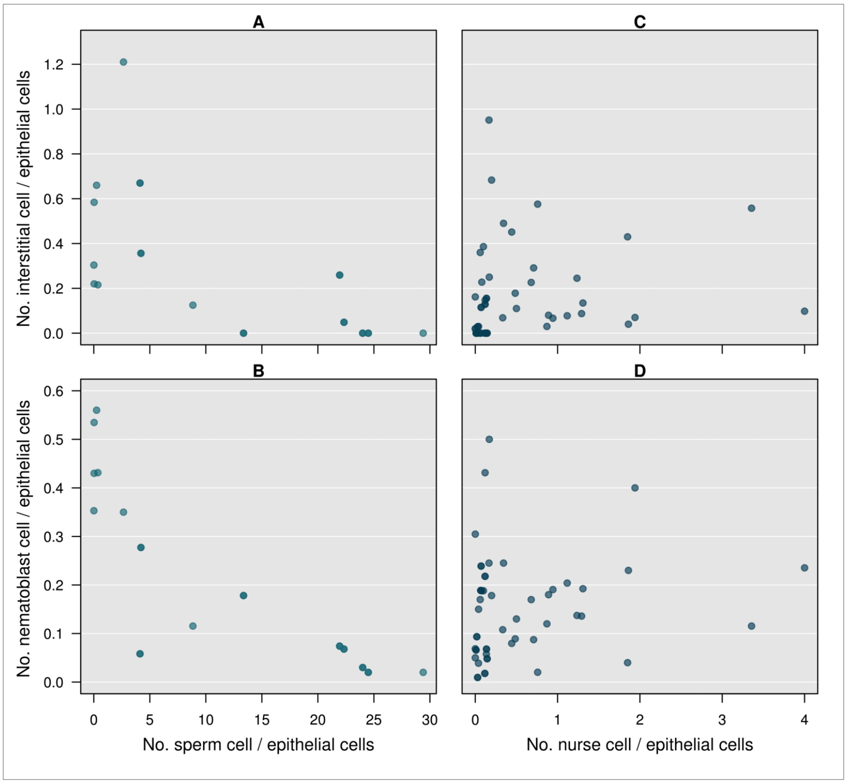

Fig. 4. Correlation between reproductive cell number (sperm or nurse cells) and interstitial and nematoblast cell number in males (a and b) and interstitial and nematoblast cell number in females (c and d). All cell numbers were normalized to epithelial cell number. Darker colour intensity refers to overlapping data points.

Discussion

In this study we scored regeneration rate and cellular composition (nematoblast, interstitial and germ cell counts) of *H. oligactis* polyps differing in reproductive strategies. We found that sexual (but not asexual) reproduction is associated with reduced regeneration ability and lower relative number of interstitial stem cells and nematoblasts involved in food capture. From an ultimate (evolutionary) perspective, the reduced somatic performance of sexually reproducing hydra might be explained by selection acting on current reproduction as opposed to long-term survival. Evolutionary theories of senescence suggest that, when extrinsic mortality risk is high, mutations that are detrimental to survival can become fixed, either because genes acting late in life after reproduction are not "visible" to selection (the "mutation accumulation" theory of aging; Medawar 1952), or because some of these mutations might increase reproduction at the expense of survival (the "antagonistic pleiotropy" and "disposable soma" theories of aging; Williams 1957; Kirkwood & Rose 1991). In natural hydra populations inhabiting the temperate zone, the onset of winter is likely to be a major mortality factor, especially in shallow waters where the risk of freezing is higher. Under such conditions, selection might act to increase reproductive investment and production of resting eggs, which are more likely to survive adverse conditions, even at the expense of diverting all cellular/metabolic resources away from somatic maintenance. Hence, post-reproductive senescence in sexual hydra polyps could at least partly be explained by stem cell depletion caused by survival/reproduction trade-offs. By contrast, since asexual buds have high mortality risk just as the parent animal, they are likely to have a lower reproductive value during autumn than resting eggs. This, in turn would select for reduced investment into sexual offspring and, consequently, lower physiological cost of reproduction. Hence, asexuals in this population might follow a strategy of producing offspring with low reproductive value at a low cost, as opposed to sexuals, which produce offspring of high reproductive value at a high physiological cost. This latter might be considered a case of terminal investment (Williams, 1966; Clutton-Brock, 1984). It remains to be shown whether these two strategies face different risk of mortality, as predicted by evolutionary theories of senescence, e.g. because of differences in individual condition, microhabitat, or any other factor, or they are a form of bet-hedging: stochastic switching between phenotypic states – a way of adaptation to fluctuating and unpredictable environments, e.g. Cohen, 1966.

Our results lend support to the hypothesis that life history decisions in *Hydra* might be mediated by a competition for a limited stem cell pool involved in multiple life functions (Rinkevich, 1996). In animals with high tissue plasticity, the activity of multipotent stem cells is required for multiple life functions. Reduced availability of stem cells has been suggested to be involved in the determination of *Tubularia* hydrant lifespan (Tardent, 1963), and also thought to be responsible for post-reproductive degeneration in *H. oligactis* (Brien, 1966; Tardent, 1968; Tardent, 1974; Bosch, 2009). Increased commitment of stem cells into germ cells likely reduce the differentiation of stem cells into somatic derivatives (a process termed "gametic crisis"; Brien, 1966; Bosch, 2009), possibly causing a decline in survival of *H. oligactis* (Yoshida, Fujisawa, Hwang, Ikeo & Gojobori, 2006). Our observation that the number of interstitial cells and nematoblasts were reduced, while germ cell numbers increased during sexual reproduction in animals from a natural population are in accordance with findings obtained under laboratory circumstances in this species (Yoshida, Fujisawa, Hwang, Ikeo & Gojobori, 2006). Although other *Hydra* species do not seem to show similar patterns of senescence, there is evidence that similar exhaustion might occur in *Aurelia* polyps that have been stimulated to strobilate (generate sexual medusa) many times (Gold & Jacobs, 2013).

In parallel to the decline in stem cell pools, regeneration rate was also reduced in sexual polyps. Trade-offs and allocation strategies between regeneration and other life history traits such as sexual reproduction, asexual reproduction, growth or successive regeneration events were reported previously (Gross, 1925; Kanajew, 1926; Tardent & Tardent, 1956; Tardent, 1963, Campbell, 1967, Rinkevich & Loyla, 1989, Martínez, 1996, Zattara & Bely 2013). Regeneration is a somatic function that likely depends on the availability of cellular resources (stem cells). Stem cells are crucial in all types of regeneration either because they proliferate to produce cells that will be involved in regeneration or because they migrate to the wound site to re-form lost body parts (Sánchez Alvarado, 2000; Bely & Nyberg, 2010; Sugimoto, Gordon & Meyerowitz, 2011). Any physiological process that reduces the availability of stem cells can therefore, in theory, limit regeneration (Kramarsky-Winter & Loya, 2000; Henry & Hart, 2005). Indeed, reduced availability of cellular resources has been invoked previously to explain the reduction in regeneration ability in *Hydra* in response to subsequent amputations (Gross, 1925; Kanajew, 1926; Tardent & Tardent, 1956; Tardent, 1963, Martínez, 1996) and the suppression of regeneration after sexual reproduction (Campbell, 1967 or vice versa: Rinkevich & Loyla, 1989).

The trade-off between differentiation into somatic and reproductive functions is further underscored by our observation that interstitial stem cell numbers and nematoblast numbers were negatively related to germ cell counts in males. Such a negative relationship could arise because individuals with a higher reproductive investment (larger germ cell counts) require large number of stem cells differentiating into the germline pathway, resulting in interstitial cell / nematoblast depletion relative to non-reproductives. Interestingly, we observed no relationship between reproductive cell numbers and interstitial cell / nematoblast counts in females. This could mean that the trade-off between germ cells and somatic cell types concern different mechanisms in females and males. However, it might also have been caused by differences in the way reproductive investment is estimated from reproductive cell counts. Specifically, in females several nurse cells become incorporated into each developing egg, and during egg maturation fewer and fewer nurse cell will be located separately (Zihler, 1972; Miller, Technau, Smith & Steele, 2000). As a consequence, females would show a progressive reduction in both nurse cell numbers and depletion of interstitial stem cells / nematoblasts during egg maturation if stem cells differentiate into nurse cells and these become incorporated into eggs. This could explain the relatively high number of females in which nurse cells, interstitial cells and nematoblasts were all depleted (Fig. 4).

In spite of the strongly reduced stem cell numbers in sexually reproducing polyps (which suggest that interstitial cells are converted to germ cells during gonadogenesis), the exact explanation for interstitial cell depletion during sexual reproduction in *H. oligactis* is still not clear. Current models of germ cell specification suggest that there are two interstitial stem cell lineages in *Hydra*, which are morphologically indistinguishable (reviewed in Nishimiya-Fujisawa & Kobayashi, 2012). Multipotent stem cells (MPSCs) give rise to somatic cells, like nerve cells, gland cells and nematocytes, while germline stem cells (GSCs) are unable to produce somatic cells but differentiate into nurse cells and sperm. GSCs derive from MPSCs (Nishimiya-Fujisawa & Kobayashi, 2012), hence MPSCs are able to produce both somatic and reproductive cells. This is supported by observations that (1) *H. oligactis* polyps in which GSCs were experimentally ablated are still able to quickly develop germ cells when exposed to cold temperature (Littlefield, Dunne & Bode, 1985) and (2) single interstitial cells in another *Hydra* species (*H. magnipapillata*) can give rise to both somatic and germline cells (Bosch & David, 1987). Because of the complexity of interstitial stem cell lineages in hydra, the gaps in knowledge of their dynamics and the indistinguishability of the two major stem cell types, it is possible that only a subset of the cells identified in this study as interstitial cells (MPSCs) take direct part in the somatic-reproductive trade-off. However, since these MPSCs can differentiate into GSCs (or directly into germ cells), the trade-off in stem cell differentiation between somatic and reproductive functions remains the same. Future studies of stem cell differentiation during gonadogenesis and hydra germline stem cells would help to elucidate the exact mechanisms behind stem cell depletion during gametogenesis observed in this species.

An alternative (or additional) explanation for the reduced number of interstitial cells, nematoblasts and lower regeneration ability of sexuals could be a depletion in resource pools in association with production of reproductive cells. If gamete production drains energy or nutrient reserves, this could limit self-renewal ability of stem cells, ultimately constraining the number of somatic cells. The contribution of energy/nutrient limitation to the observed trade-off between stem cells and reproductive cells could be significant, since otherwise stem cells might be able to renew themselves indefinitely, even during sustained gametogenesis. Conversely, if the mortality rate of sexual individuals is high, there might be relaxed selection for self-renewal ability and stem cells might not replace themselves even if experiencing high resource availability. A more detailed description of differentiation patterns and changes in self-renewal capacity of interstitial cells during sexual development would possibly help to elucidate these questions. While sexual reproduction was associated with reduced somatic cell types and regeneration ability, this reduction seemed to be lower in asexual individuals. Interestingly, this pattern mirrors the phylogenetic distribution of reproductive mode and regeneration ability in several invertebrate groups: regenerative capacities are often higher in species capable of asexual reproduction in segmented worms (Zattara & Bely, 2016) and flatworms (Peter, Ladurner & Rieger, 2001), relative to obligately sexual ones. The higher stem cell pools and regenerative potential of asexual polyps indicates that this type of reproduction might impose different cost on the parent polyp compared to sexual reproduction. Alternatively, the reduced somatic cell number and regenerative ability of asexuals might have been the result of higher physiological condition of asexuals compared to sexuals. In many organisms capable of both sexual and asexual reproduction, theory predicts that the latter might be more prevalent under benign conditions and high food availability (Sakai 1995; Gardner & Mangel 1999). If reproductive mode of hydra depends on food availability as predicted by theoretical models, then individuals experiencing high food availability would be less likely to undergo sexual development and concomitantly also experience improved physiological condition, higher cell proliferation rate and more cellular resources available for maintaining somatic tissues and regeneration ability. Increased cell proliferation rate in response to high food availability has indeed been documented in several animal groups (e.g. Bosch & David 1984, Zattara & Bely 2013). However, in a previous laboratory study of *H. oligactis* individuals from this population (Tökölyi, Ősz, Sebestyén & Barta, 2017) we did not find an effect of food availability on reproductive mode, which contradicts this hypothesis (although other aspects of the environment, such as its predictability, or the presence of abiotic stressors or parasites need to be taken into account to unequivocally answer this question). Lastly, another explanation for the marked differences between sexual and asexual individuals might lie in the fundamentally different ways in which development proceeds in the two strategies. Specifically, asexual offspring in hydra are largely formed of stem cells which can replenish themselves and possibly contribute to the somatic maintenance of the parent. Conversely, stem cells destined for gamete production are irreversibly committed to this path and therefore unusable in somatic maintenance. This irreversible commitment could create a ratchet of reduced somatic maintenance and deteriorating condition and, ultimately a point of no return for the parent animal.

In addition to describing patterns of reproductive mode, stem cells and regeneration in *H. oligactis*, we also provide data on the natural phenology of sexual reproduction for this species. While gametogenesis in *H. oligactis* is known to occur during the autumn and to last until early winter, the ecology of *H. oligactis* has been investigated by only a handful of studies so far (Welch & Loomis, 1924; Miller, 1936; Bryden, 1952; Reisa, 1973; Ribi, Tardent, Tardent & Scascighini, 1985). Previous studies have shown that, in general, only a subset of the population reproduces sexually at any time; moreover, sexually reproducing animals are not found in some years (Miller, 1936; Bryden, 1952; Ribi, Tardent, Tardent & Scascighini, 1985). In this Hungarian population, the proportion of sexually reproducing individuals was also lower than that of agonadic individuals (Fig. 1). Interestingly, the proportion of asexual animals showed an increasing tendency towards the onset of winter (even though the temperature was decreasing), possibly because sexual individuals were disappearing or reverted back to asexual reproduction, which is known to occur in individuals collected from this population under laboratory conditions; (Tökölyi, Ősz, Sebestyén & Barta, 2017). Together with the observations that (1) initiation of sexual reproduction in *H. oligactis* strongly depends on the rate of the temperature drop (Kaliszewicz, 2015) and (2) some *H. oligactis* strains seem to have a lower propensity to initiate sexual reproduction (Tökölyi, Ősz, Sebestyén & Barta, 2017, these observations suggest that sexual reproduction in *H. oligactis* could be a conditional and/or polymorphic strategy.

Overall, our results suggest that sexual reproduction imposes a high physiological cost on *Hydra oligactis* polyps. The reduced regeneration abilities and depletion of stem cells in sexually reproducing animals compared to non-reproductives might imply that current sexual reproduction is an irreversibly induced reproduction strategy and gamete production is prioritized over the maintenance of somatic functions and future survival during autumn. The highly divergent life history decisions of *H. oligactis* provide a great model system to study aging and non-senescent life history tactics and its physiology within a single species. In addition, in order to clarify the role of limiting cellular factors, further studies focusing on common cellular pools required by life history traits are much needed.

Authors' Contributions

FS, ZB & JT conceived research, FS & JT collected and analysed data. FS, ZB & JT wrote the manuscript.

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Data Accessibility

Data for this paper have been archived in figshare: https://doi.org/10.6084/m9.figshare.6449579.v1.

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4.2. 2. tanulmány / Study 2: Age-dependent plasticity in reproductive investment, regeneration capacity and survival in a partially clonal animal

Age-dependent plasticity in reproductive investment, regeneration capacity and survival in a partially clonal animal

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Abstract

1. Asexual reproduction diversifies life history priorities and is associated with unusual reproduction and somatic maintenance patterns, such as constant fertility with age, extensive regeneration ability and negligible senescence. While age-dependent plasticity in relative allocation to sexual vs. asexual reproductive modes is relatively well studied, the modulation of somatic maintenance traits in parallel with age-dependent reproduction is much less well understood in clonal animals.

2. Here, we asked how age-dependent investment into sexual and asexual reproduction co-varies with somatic maintenance such as regeneration in a partially clonal freshwater cnidarian, *Hydra oligactis*. We investigated this question in *H. oligactis,* because they have remarkable regeneration abilities, experimentally inducible sex and we targeted compound measures of both reproduction and somatic maintenance at multiple organismal levels.

3. We induced sexual reproduction by lowering temperature at two ages, one week or four weeks after detachment from an asexual parent, in animals of a male and a female clone. Then we measured phenotypically asexual and sexual reproductive traits (budding rate, start day and number of sexual organs) together with head regeneration rate, survival and the cellular background of these traits (number of reproductive and stem cells) for two or five months.

4. Younger animals had higher asexual reproduction while individuals in the older group invested more into sexual reproduction and reproductive cell production. In parallel with these age-dependent reproductive differences, somatic maintenance of older individuals was also impacted: head regeneration, survival and stem cell numbers were reduced compared to younger polyps. 5. Our results highlight the role of age in a clonal species and suggest that asexual reproduction coupled with higher somatic maintenance is prioritized earlier in life, while sexual reproduction with higher maintenance costs occurs later. Our findings of age-dependent modulation of somatic maintenance and reproductive modes might provide an insight into the evolution of ageing by life history switches. The marked age-dependent changes in *H. oligactis* can be valuable for a wide range of clonal organism research and might enhance the repeatability of further experimental designs.

Key words
Eco-Evo-Devo; partial clonality; phenotypic plasticity; regeneration; resource allocation; senescence; sexual investment; stem cells

Introduction

Living in a constantly changing environment poses a challenge for organisms. The optimal phenotype is expected to change with the environment (Wallace, 1986), hence phenotypic plasticity may help ensure fitness (Boeing, Ramcharan, & Riessen, 2006). Plasticity might be especially important for clonal populations and species, because it can reduce the negative effects of low genetic variation, enable the occupancy of a wide range of habitats and allow sessile species to adapt to temporal environmental changes (Bruno & Edmunds, 1997). Theoretical studies suggest that phenotypic plasticity is favoured in varying environments, when costs of plasticity are low and reliable environmental cues are available (Fischer, van Doorn, Dieckmann, & Taborsky, 2014 and references therein). Cues for phenotype modifications can be both external abiotic agents such as temperature (e.g. for regeneration in corals; Lester & Bak, 1985), salinity (e.g. for reproduction in copepods; Chen, Sheng, Lin, Gao, & Lv, 2006) or space limitation (e.g. for growth in corals; Muko, Sakai, & Iwasa, 2001) or biotic factors like bacteria (e.g. for elemental composition in *Daphnia*; Frost, Ebert, & Smith, 2008), population density (e.g. for diapause in rotifers; Schröder & Gilbert, 2004) or presence of predators (e.g. for morphological defense in *Daphnia;* Tollrian, 1995).

At the same time, West-Eberhard (2003, p. 33) mentions *internal* environment as well in her definition of phenotypic plasticity “The ability of an organism to react to an internal or external environmental input with a change in form, state, movement, or rate of activity”. Internal factors affecting phenotypic plasticity comprise body size and condition including energy reserves, immune system or nutritional status, among many other traits (McNamara & Houston, 1996). Age, as an internal environmental factor, correlates with condition, reproduction and survival and is part of an organism's state (McNamara & Houston, 1996). In addition, information acquisition with age can be crucial for decision making in precarious environments (Fischer et al., 2014): if an organism does not have perfect information about its environment at birth, but can improve its estimate about the environment with time, phenotypic adjustment is expected to change with age in a nonmonotonic fashion (Fischer et al., 2014).

Allocation of resources to basic life functions often shows age- or life stage-dependent plasticity (Radchuk, Turlure, & Schtickzelle, 2013; Richardson & Smiseth, 2019). For instance, an increase in fecundity with age is common in both indeterminate and determinate growers (Berube, Festa-Bianchet, & Jorgenson, 1999; Clutton-Brock, 1984; Martin, 1995), because when growth stops or declines after maturity, it is optimal to direct the remaining resources into reproduction (Cichoń, 2001). Repair intensity of somatic damage (i.e. somatic maintenance) also varies with age but generally declines after maturity, being highest early in life, diminishing later and stopping completely well before the end of the maximum expected life (Cichoń & Kozłowski, 2000). Empirical evidence confirms this pattern in many taxa (e.g. reviewed in: Jones et al., 2013; Ricklefs, 2008) and the decline in somatic maintenance is a decisive component in theories of senescence such as the "antagonistic pleiotropy" or "disposable soma" hypotheses (Kirkwood & Rose, 1991). Due to competition over limited energy or nutrients, age-dependent allocation strategies can shape all main life history components responsible for growth, reproduction, somatic maintenance or survival by trade-offs between them (Kozłowski, 1992, but see Cox, Lovern, & Calsbeek, 2014). The extent of resource limitation can be reduced via resource acquisition thus "high-quality individuals" can show less trade-offs, but resource acquisition also varies with an individual's life cycle and be more determinant for resource allocation at early ages (Richardson & Smiseth, 2019; Yearsley et al., 2005).

Age-dependent allocation decisions to reproductive and somatic maintenance functions can be much more complex in clonal species. Resource allocation priorities of clonal animals in certain life stages can differ from what is expected in non-clonal species (Clutton-Brock, 1984; Engen & Saether, 1994; Stearns, 1992) and the integration of clonal growth into life-history models is complicated (Gardner & Mangel, 1999). For instance, resource allocation models of conventional life histories predict an optimal strategy of growing early, then stopping growth and starting reproduction (Cichoń, 1997). However, empirical studies of clonal animals detect a different pattern: clonal reproduction can be more emphasized in early life in the highest condition and growth is more important later (Glazier & Calow, 1992). Another major life history decision in partially clonal species is the switch from asexual to sexual reproduction, which might be beneficial when clonal reproduction is limited by exogenous factors and triggered later in life as condition deteriorates with age (Burke & Bonduriansky, 2018; Harvell & Grosberg, 1988).

While the reciprocity of asexual and sexual strategies has been relatively well studied in partially clonal organisms, it is still unclear how somatic maintenance co-varies with these age-dependent reproductive allocation decisions. In partially clonal animals asexual forms often have higher somatic maintenance functions, such as higher regeneration ability (Krois, Cherukuri, Puttagunta, & Neiman, 2013; Saccucci, Denton, Holding, & Gibbs, 2016), increased telomerase activity (Tan et al., 2012), and - possibly as a consequence of these - a lower rate of senescence, such that some of them (e.g. *Hydra* species; Schaible et al., 2015) are potentially immortal (reviewed in Sköld & Obst, 2011, but see Martinez & Levinton, 1992; Sköld, Asplund, Wood, & Bishop, 2011). Despite the exceptional somatic maintenance abilities of clonal animals, these traits are not constant during their lifetime (for instance, telomerase activity (Sköld et al., 2011), regeneration (Meesters & Bak, 1995) or survival (Orive, 1995) decrease with ageing). However, information is scarce about how resource allocation between reproductive modes and regeneration or any other somatic maintenance trait varies with age in clonal organisms. This lack of knowledge hinders understanding senescence in clonal or partially clonal species that often exhibit unconventional, complex senescence patterns such as negligible senescence (constant mortality and fecundity with age) or even negative senescence (decreasing mortality and increasing fecundity with age) (Vaupel, Baudisch, Dölling, A. Roach, & Gampe, 2004).

Here, we studied age-dependent plasticity in both reproductive investment (sexual and asexual reproduction) and somatic maintenance (regeneration capacity and survival rate) in a partially clonal freshwater cnidarian, *Hydra oligactis*. Hydra polyps have exceptional regeneration capacity (e.g. whole body regeneration from a piece of tissue as small as 1% of the normal polyp; Shimizu, Sawada, & Sugiyama, 1993) that has captured the imagination of scientists since the 18th century (Trembley, 1744). Regeneration research in hydra has focused on the physiological, developmental and molecular processes underlying this phenomenon (e.g. Bode, 2003; Schaller, 1976; Tardent, 1974) but regeneration as a life history trait and its age-dependent modulation are less well understood. Regeneration provides obvious advantages via eliminating the negative effects of crucial body part injuries, which is especially important when the probability of repeated injuries is high (Bely & Nyberg, 2010). But as many other traits - like growth or reproduction - regeneration also requires resource investment, thus may impair other life history traits or vice versa (Henry & Hart, 2005) .

Regeneration ability in *H. oligactis* is a highly plastic trait, e.g. it is impaired in sexual individuals of *H. oligactis* (Sebestyén, Barta, & Tökölyi, 2018; Tomczyk et al., 2017) Loss of regeneration in this species may be a consequence of their unique life history within the genus *Hydra*: they switch from asexual reproduction to sexual reproduction during the autumn, in order to produce a relatively high number of sexual organs and resting eggs (compared to other *Hydra* species: Schuchert, 2010) then the polyps regularly degenerate and die (Schenkelaars et al., 2017; Tökölyi, Ősz, Sebestyén, & Barta, 2017; Yoshida, Fujisawa, Hwang, Ikeo, & Gojobori, 2006). Post-reproductive senescence is accompanied by drastic changes in cellular composition: the number of interstitial stem cells declines while the number of reproductive cells increases (observed in laboratory strains: Tardent, 1974; Yoshida et al., 2006 and animals derived directly from their natural habitat: Sebestyén et al., 2018). Depletion of stem cells suggests that stem cells differentiate into reproductive cells and limit somatic maintenance functions (Yoshida et al., 2006). Importantly, a recent population genetic analysis indicated substantial phenotypic plasticity in reproductive strategies in this species under field conditions (Miklós et al., 2019), but the factors inducing this plasticity are unclear.

We used clonal offsprings of a male and a female *H. oligactis* polyp to examine the role of age in life-history trait plasticity. We asked whether investment into different reproductive modes and somatic maintenance traits changes with age. *Hydra oligactis* is an ideal species for investigating age-dependent cost of reproductive modes, because they can be experimentally induced to reproduce sexually by lowering temperature. We induced sexual reproduction by temperature change and measured several aspects of both reproduction and somatic maintenance for eight weeks after induction of sexual reproduction in two age groups (1 week and 4 weeks old): the number of reproductive organs (eggs and testes), start of sexual reproduction along with asexual reproduction, head regeneration ability and survival. The switch between asexual and sexual reproduction can be affected by their stem cell demands: asexual reproduction requires proliferation of the continuously maintained somatic stem cells (which are responsible for somatic maintenance in general; Sköld, Obst, Sköld, & Åkesson, 2009), while sexual reproduction requires production of germ cells formed from undifferentiated stem cells, usually occurring after some asexual cycles and time for maturation (Harvell & Grosberg, 1988; Simon, Rispe, & Sunnucks, 2002). Hence, we also investigated the cellular requirements for these functions: the number of reproductive cells as a measure of sexual reproductive investment, and the number of somatic interstitial stem cells as a requirement for somatic maintenance and asexual reproduction. Our experimental framework provides not just an insight into how animal reaction to the same environmental cue can change with age, but investigates the complexity of changes and organismal levels involved.

If fecundity increases and somatic maintenance decreases with age (as general life history theory suggests), we expect more sexual organs and reproductive cells in older animals, while their regeneration ability, survival and stem cell number should be reduced. Conversely, it is also possible that both fecundity and somatic maintenance is higher in older individuals because these might have more time to accumulate sufficient resources to sustain both life functions. Asexual reproduction might be prioritized in younger animals in accordance with observations on other partially clonal animals (e.g. Harvell & Grosberg, 1988), although it might show an opposite pattern due to higher condition of adults.

Materials & Methods

Hydra strains, culture conditions and experimental design

Experimental animals originate from an oxbow lake near Tiszadorogma in Eastern Hungary (47.6712N, 20.8641E) and have been kept in the laboratory as culture strains for one year prior to the experiments described here. We kept animals individually in 6-well tissue culture plates under standardized conditions in a climate chamber (18 °C temperature, 12/12 h dark/light photoperiod) and ~ 5 ml standard hydra medium (1.0 mM $CaCl_2$, 0.1 mM $MgCl_2$, 0.03 mM KNO_3, 0.5 mM $NaHCO_3$, 0.08 mM $MgSO_4$; Sebestyén et al., 2018). Sexual reproduction was induced by moving plates with animals to a wine cooler with even air flow on 7 °C and 8 h light / 16 h dark photoperiod. We fed the hydras with 20 µl freshly hatched *Artemia spp.* nauplii (Tökölyi et al., 2016) two times a week, on the same days. We changed hydra medium on feeding days and on days after feeding (for removing food remains).

We used one male (Number of animals (N) = 666) and one female strain (N = 660) for our experiments, in which freshly detached buds were used 0-4 days after their detachment. Experimental treatment consisted of moving polyps to cold temperature at either of two different time points: 3 days or 24 days after their initiation (Fig. 1). Because of the variation in the detachment time, these time intervals resulted in a maximum possible age of 7 days (henceforth called “1 week” group) or 28 days (“4 week” group). We used different animals for different types of measurements (head regeneration, composition and survival). We measured both head regeneration and cell number 8 times (weekly for 2 months) after cooling. For logistical reasons, experimental animals were initiated in several distinct batches.

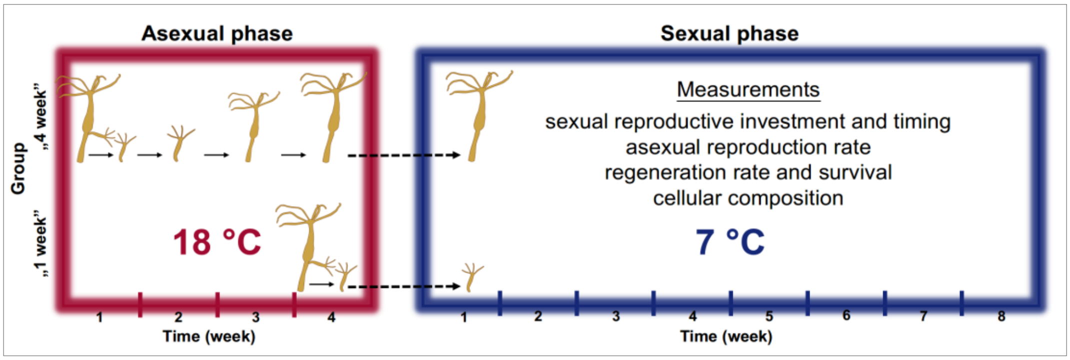

Fig. 1, Experimental design containing two groups: 1 week or 4 week old according to their age at cooling. Following the temperature change, we measured the number of sexual organs and their first appearance, asexual reproduction rate, head regeneration rate and cellular composition (reproductive and stem cell number) for two months, and survival rate at the end of the fifth month after cooling. Experimental animals were propagated asexually.

Detection of sexual organs and asexual reproduction rate

Hydra oligactis males spread their sperm from several separate testes on their body column, and females produce eggs which detach after maturation (Reisa, 1973). Sexual reproduction in both sexes is continuous during the sexual period. We used a stereo microscope to count number of testes on one side of males two times a week and number of detached eggs in females four times a week (each time the medium was exchanged), following temperature change. We measured sexual reproduction for two months after cooling, since all animals had started their sexual cycle by the end of the second month and repeated sexual cycle has not been reported in this species. We recorded the number of detached buds twice a week on feeding days for 20 weeks after cooling, because asexual reproduction can be repeated several times.

Head regeneration measurements

We measured head regeneration in approximately half of the animals (N = 711). We decapitated the animals below their mouth at about 15% of body length (containing the mouth tip, tentacles and 10% of the body column). We coded the presence or absence of emerging tentacles one week after decapitation (Sebestyén et al., 2018). Animals were not fed during these measurements.

Cell number measurements

We performed cell number measurements on 187 animals approximately weekly for two months after temperature change. We macerated polyps according to standard technique (David, 1973), placed 5 µl of a sample on microscopic slide, which was then examined under 400x magnification in an Euromex iScope phase contrast microscope. We recorded epithelial cell, sperm precursor/nurse cell and interstitial stem cell number (total number of 1,2,3-4, 5-8, >8 nests i.e. group of cells) for each sample. Sample measurements were made until we counted at least 100 epithelial cells and cells around them belonging to different cell types (Sebestyén et al., 2018).

In males, sperm precursor and non-mature spermatid cells were identified according to the work of Littlefield et al.(1985). We estimated sperm precursor and non-mature spermatid cell number by counting cell groups, because the large number of cells made exact counting impossible. In females, interstitial cells largely increase their size before reproductive cell formation and give rise to nurse cells (Zihler, 1972), hence in early stages of development reproductive cells are distinguishable from interstitial cells by their increased cytoplasm volume. We counted a cell as a nurse cell if the diameter of its nucleus was at least half-sized compared to the diameter of the cell or smaller. A subset of nurse cells produces gametes while the others are phagocytosed by the oocyte (Alexandrova, Schade, Böttger, & David, 2005), hence all nurse cells contribute to reproductive investment. We were not able to do histological preparations in females 8 weeks post-cooling because all individuals involved consisted of acellular necrotic tissue.

Survival measurements

We kept experimental animals for five months and recorded death when animals were fully disintegrated or disappeared. We retained hydras even if they shrank to a very small size. Animals were scored as survived if they had intact body and tentacles or produced at least one bud until the end of the fifth month. We excluded 12 animals from the analysis due to their accidental loss.

Statistical analysis

To analyze the effect of age at cooling and time after cooling on asexual reproduction rate we used Generalized Linear Models (GLMs) with Poisson error distribution (Bates, Mächler, Bolker, & Walker, 2015), since the number of detached buds was a count variable. We tested the effects of age at cooling and time after cooling separately and the interaction between them via Likelihood Ratio Tests (LRTs). We performed unpaired two-sample Wilcoxon tests to analyze timing (start day after cooling) and investment (number of sexual organs) of sexual reproduction in groups with different age at cooling.

For testing the effect of age at cooling and time after cooling on sperm precursor/nurse cell number and interstitial stem cell number we used zero-inflated models, because our data contained more zero values in some groups than it could be expected by Poisson or Negative binomial distribution (Zeileis, Kleiber, & Jackman, 2008). Our models contained cell number (sperm precursor or nurse cell, stem cell) as dependent variable and age at cooling and time after cooling as predictors. We included epithelial cell number as a fixed effect, to normalize to epithelial cell number which was not exactly identical in every sample.

We analyzed the effect of age at cooling on temporal changes in head regeneration by GLM with binomial distribution. Our model contained head regeneration (presence or absence) as dependent variable and age at cooling, time after cooling and their interaction as independent variables. We tested the effect of the two factors (age at cooling and time after cooling) and the interaction between them by LRTs. We checked the effect of start date (because animals entered into the experiment at different dates may slightly differ from each other) as random effect in models without interaction, but the results of these were consistent with the original models. For the analysis of total head regeneration data of all dates after cooling, we used unpaired two-sample Wilcoxon tests. For testing the effect of age at cooling on survival data, we performed Fisher's Exact Tests. All analyses were performed in R Statistical Environment version 3.4.4 (R Core Team, 2018)

Results

Asexual reproduction rate

Age at cooling and time after cooling had significant effects on budding rate in both sexes (age at cooling: LRT, males: d.f. = 1, p < 0.001, females: d.f. = 1, p < 0.001; time after cooling: LRT, males: d.f. = 19, p < 0.001, females: d.f. = 19, p < 0.001). The interactions between these effects were also significant (LRT, males: d.f. = 19, p < 0.001, females: d.f. = 19, p < 0.001). Based on the overall data of the 20 weeks, animals belonging to the "1 week" group had significantly higher budding rate (Wilcoxon test, males: N = 4469, p < 0.001, females: N = 4348, p < 0.001). As Fig. 2 presents, budding rate showed an increasing tendency in all groups during the experiment.

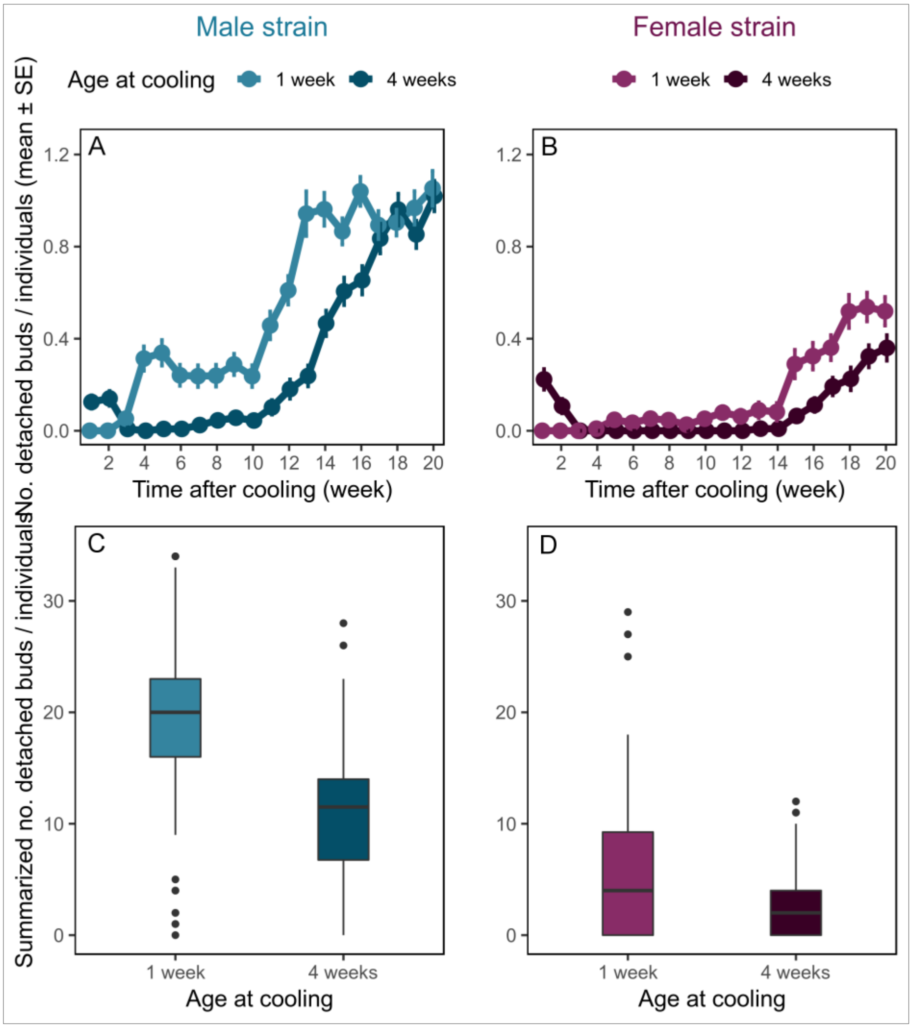

Fig. 2, Weekly mean numbers of detached buds (A and B) and the overall weekly mean numbers (C and D) in two age groups in two stains.

Sexual investment and timing

Males in the "1 week" group had significantly fewer testes (Wilcoxon test, N = 107, p < 0.001) and initiated sexual reproduction significantly later (Wilcoxon test, N = 107, p < 0.001), than males which were 4 weeks old at cooling (Fig. 3). Females in the "1 week" group had significantly fewer detached eggs (Wilcoxon test, N = 112, p < 0.001) and delayed sexual reproduction (Wilcoxon test, N = 112, p < 0.001) compared to 4 weeks old females at cooling.

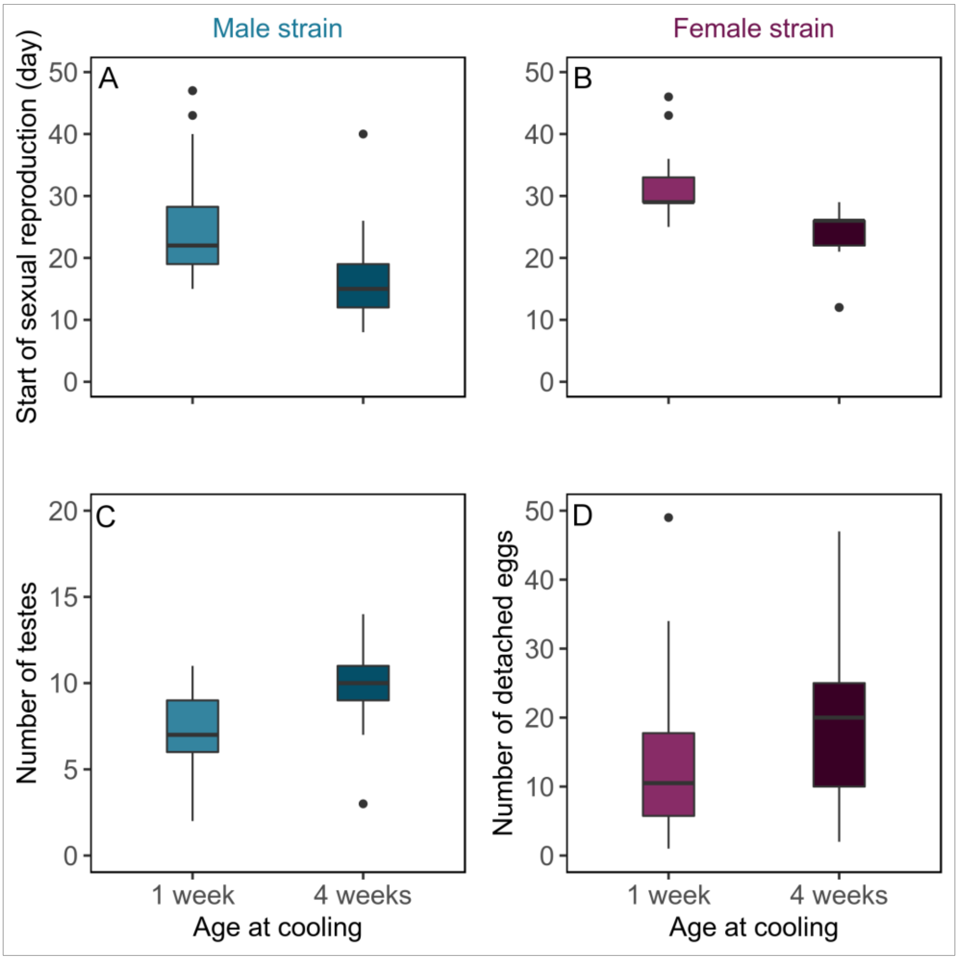

Fig. 3, Start day of sexual reproduction (male strain (A), female strain (B)) and the overall number of sexual organs (testes in the male strain (C), detached eggs in the female strain (D)).

Head regeneration

Age at cooling and time after cooling significantly affected head regeneration in both sexes (age at cooling: LRT, males: degree of freedom = 1, p = 0.034, females: d.f. = 1, p < 0.001; time after cooling: LRT, males: d.f. = 1, p < 0.001, females: d.f. = 1, p < 0.001). The interaction between age at cooling and time after cooling had no significant effect on head regeneration in males (LRT, d.f. = 1, p = 0.75) or females (LRT, d.f. = 1, p = 0.69). Overall, both older males and females had significantly lower regenerative ability than younger males and females respectively (Wilcoxon test, males: N = 347, p < 0.001; females: Wilcoxon test, N = 364, p < 0.001, Fig. 4)

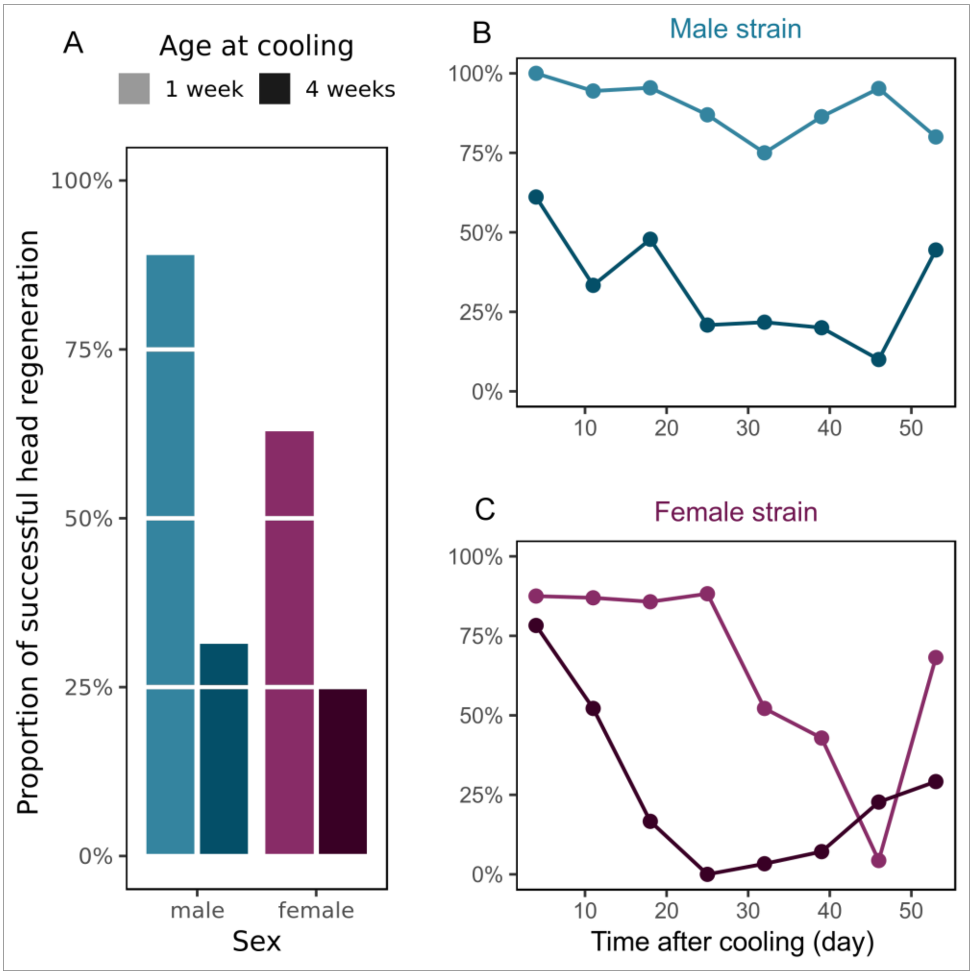

Fig. 4, Overall successful head regeneration rate in the two strains (A) and the proportions of weekly measurements in the male strain (B) and the female strain (C).

Survival

Polyps in the “1 week” group had significantly higher proportion of fully-recovered individuals five months after cooling in males (Fisher test, p = 0.002) and females as well (Fisher test, p = 0.028, Fig. 5).

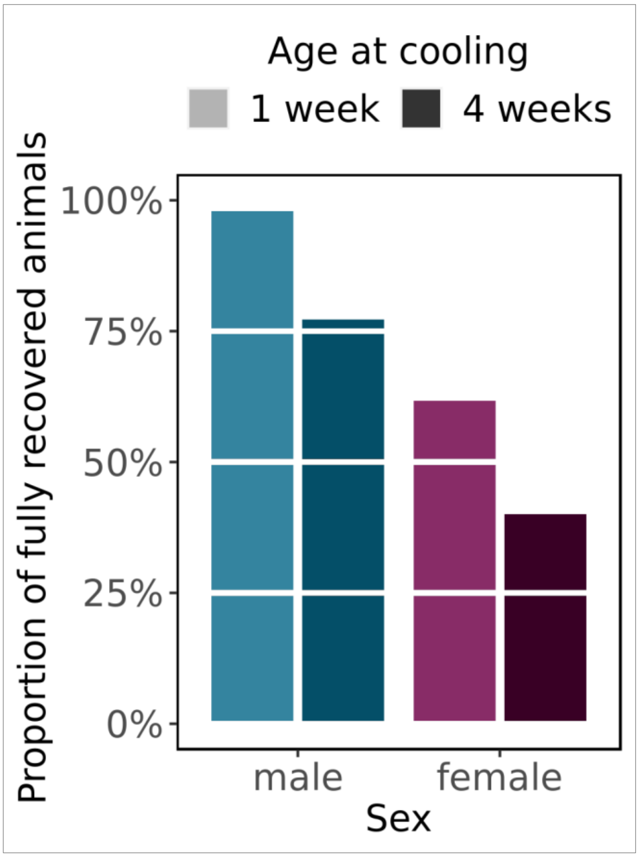

Fig. 5, Proportion of fully recovered animals following sexual reproduction at the end of the fifth month after cooling. Animals were recorded as survived if they had fully intact bodies and tentacles or if they produced at least one bud after sexual reproduction until the end of the experiment.

Sexual reproductive cell number

In isolation, age at cooling had significant effect only in males (Vuong test, in males: z-score = 1.483, p = 0.023, in females: z-score = 0.472, p = 0.318) and time after cooling had no significant effect in either sex (Vuong test, in males: z-score = 1.287, p = 0.071, in females: z-score = 1.075, p = 0.141). However, the interaction between these two were highly significant in males (Vuong test, z-score = 2.256, p = 0.014) and females (Vuong test, z-score = 1.849, p = 0.032). In all sexes, reproductive cell numbers showed a delayed start of sexual reproduction in animals which were younger at cooling (Fig. 6).

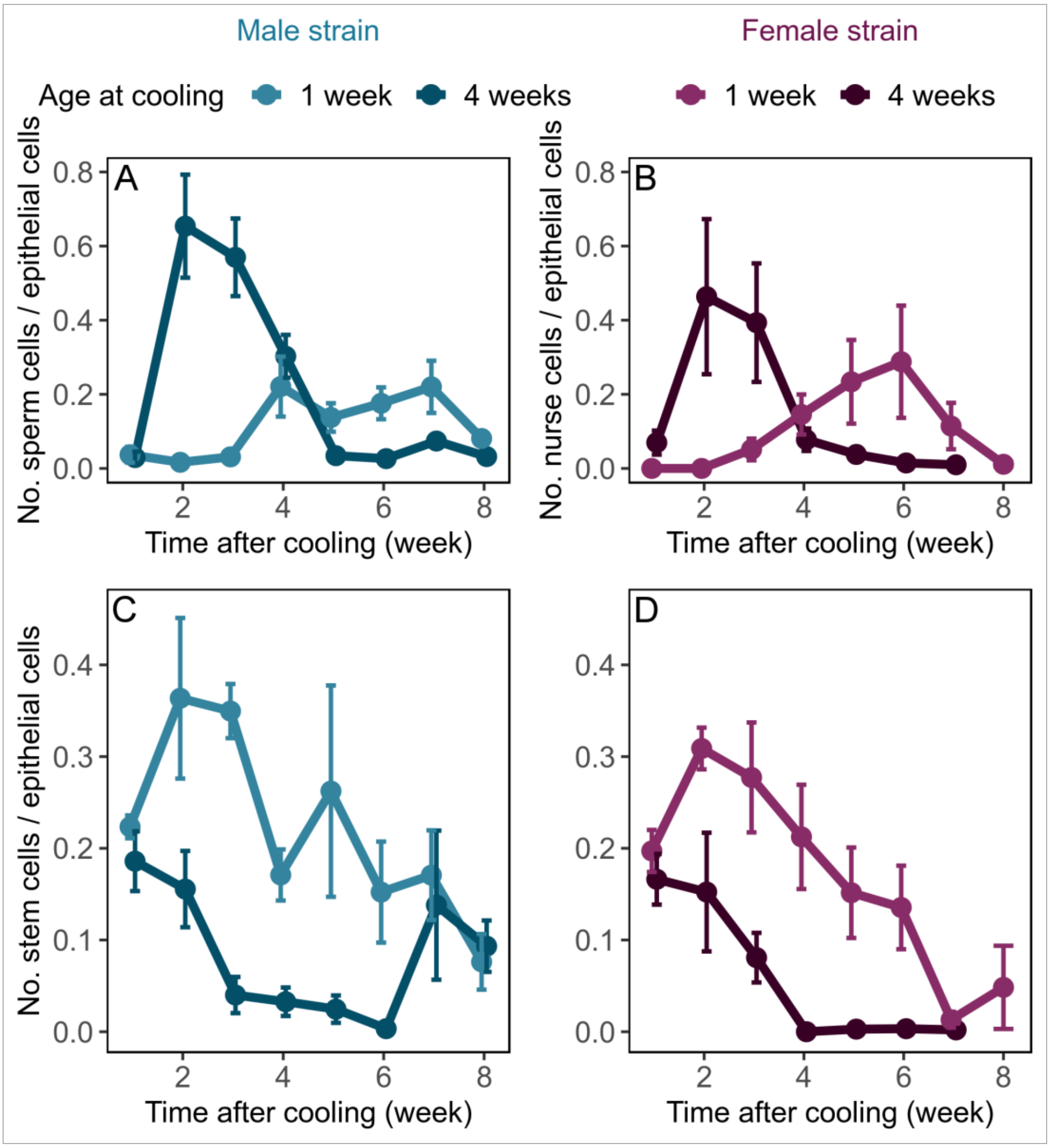

Fig.6, Weekly measurements of reproductive cells (sperm precursor or nurse cells, A and B) and stem cells (C and D) in the two age groups in two strains.

Interstitial stem cell number and age

In case of males, age at cooling had significant effect on the number of stem cells (Vuong test, z-score = 2.883, p = 0.002) and time after cooling had a marginally significant effect on male stem cell number (Vuong test, z-score = 1.419, p = 0.078). There was no significant interaction between the effect of age at cooling and time after cooling in males (Vuong test, z-score = 0.594, p = 0.276).

In females, the effect of age at cooling and time after cooling had significant effect independently (age at cooling: Vuong test, z-score = 2.436, p = 0.007; time after cooling: Vuong test, z-score = 3.306, p < 0.001). The interaction between age at cooling and time after cooling was marginally significant in case of females (Vuong test, z-score = 1.535, p = 0.062). Stem cell number of males and females showed a decreasing tendency in both age groups during the weeks at cool temperature, as Fig. 6 indicates.

Discussion

Here, we demonstrated that age has a clear effect on life-history traits in a clonal freshwater cnidarian. We found that younger hydra polyps of the same clone had reduced and delayed sexual reproduction while they had higher asexual reproduction (budding). These differences in reproductive strategies were paralleled by somatic maintenance patterns: younger polyps had higher head regeneration rate, stem cell numbers and survival rate. Our observations indicate that sexual investment is plastic and influenced by age in hydras and these reproductive decisions impact the cost of reproduction in this partially clonal animal.

Age might influence animal reaction to environmental changes via information acquisition or optimal resource allocation patterns (Fischer et al., 2014; Radchuk et al., 2013), although the complex age-dependent plasticity in clonal organisms is vaguely understood. Age or life stage may affect how allocation to reproduction, regeneration, growth or maintenance is prioritized, but there is a difference in this nexus between non-clonal and clonal organisms. In a clonal organism growth is not just the increase in size of a single somatic unit, but it is the iteration of the unit as well (which can sexually propagate in the future) (Harvell & Grosberg, 1988). Asexual reproduction is thought to maintain a population of locally adapted clones (Ayre & Miller, 2004) and less costly than sexual reproduction (Rispe, Pierre, Simon, & Gouyon, 1998). For this reason, asexual reproduction can be advantageous for animals in good conditions and early in their life, since they can rapidly colonize resources for their own genotype, while later they can use more costly sexual reproduction to increase genotypic diversity (Burke & Bonduriansky, 2018).

Our study confirms that sexual reproduction is prioritized later in life in *H. oligactis*. This is in accordance with previous findings in other clonal species. For instance, female stick insects mated in early life (prior to the start of parthenogenetic reproduction) produce fewer eggs, possibly because they were not fully mature at pairing (Burke & Bonduriansky, 2018). Age-dependent sexual maturity is also known in some coral species (Kai & Sakai, 2008), while in *Pelmatohydra robusta* (likely synonymous to *H. oligactis*; Schwentner & Bosch, 2015) a decreased sexual maturation time and increased fecundity with polyp age were reported previously by Noda (1982). Age at sexual maturity can be also affected by body size, because age might be its predictor and in long-lived clonal animals there is often a minimum size for sexual reproduction (Harvell & Grosberg, 1988). Body size might have played a role in our experiment as well, because the importance of growth is known in another hydra species (*H. vulgaris,* in which growth occurs during the first three weeks even in starvation; Levitis & Goldstein, 2013) and the early investment into growth could set back sexual reproduction in our study species. Our study also confirms that asexual reproduction in *H. oligactis* is more important at a younger age, paralleling other partially clonal animals where asexual cycles usually forgo sexual cycles (Olive, 2002) (e.g. in rotifers: Denekamp et al., 2009, planaria: Castle, 1927 and ascidians: Gasparini et al., 2015). Although these general age-dependent reproductive mode patterns are known, experimental induction of reproductive mode switch in different ages is less frequently investigated but is needed to explicitly assess the costs and benefits of switching to sex.

We found that these age-dependent reproductive modes are associated with different somatic maintenance costs: youngs with higher asexual reproduction, delayed and reduced sexual reproduction had higher regeneration and survival, while the opposite was true for older hydra polyps. In other clonal species, high regeneration ability also often associates with asexual reproduction capabilities and higher/increasing survival rate (Zattara & Bely, 2016; Mouton, Grudniewska, Glazenburg, Guryev, & Berezikov, 2018), while the more costly sexual reproduction usually impairs these traits (Harvell & Grosberg, 1988; Henry & Hart, 2005). The negative effect of sexual reproduction is more emphasized when it is usually induced by stress and harsh conditions (Harvell & Grosberg, 1988), thus the response and investment is more urgent and intense (hypothesized in the study species *H. oligactis* as well e.g. Tardent, 1974), although these relations are generally not studied simultaneously with age-dependent changes. Although somatic maintenance traits as regeneration and survival usually do not decline with age in clonal organisms (Tanner, 2001; Yun, 2015), opposite patterns are observed in some cases (e.g. Meesters & Bak, 1995; Orive, 1995; Sköld et al., 2011; Vitt & Cooper Jr., 1986). This may further suggests that predicting age-related somatic maintenance changes and senescence patterns depend on other life-history parameters of the organism as well (Orive, 1995), like the mode of reproduction.

On the proximate level, the increasing sexual propensity with age and the higher costs of reproduction in older polyps could be explained by the differentiation patterns of the underlying cell lineages. Reproductive cells in hydra are produced by germline stem cells (a stem cell lineage that is morphologically very similar to multipotent stem cells but is restricted to gamete production; (Nishimiya-Fujisawa & Kobayashi, 2012). Germline stem cells derive from multipotent somatic stem cells that also give rise to somatic derivatives such as nematocytes, gland cells and nerve cells. Hence, sexual maturation might result in a shift in the differentiation of multipotent stem cells from somatic to reproductive derivatives. There are some observations indicating that the number of germline stem cells might increase with age after detachment from the asexual parent, at least in females (Littlefield, 1991). These indicate a preparedness for sexual reproduction in terms of the number of germline stem cells in older age, and it is possible that there are not enough germline stem cells to produce a high number of reproductive cells in one week old polyps. The cost of sexual reproduction could emerge from the reduced availability of limited amount of multipotent stem cells and their somatic derivatives, which are necessary for asexual reproduction, regeneration and somatic maintenance in general (Nishimiya-Fujisawa & Kobayashi, 2012). The sex of polyps can make this phenomenon more complicated, and although we did not compare males and females since each sex was represented by only one clone, some differences between the male and female clones were present.

The presence of age-dependent plasticity in *H. oligactis* could explain some of the variation in life history strategies observed in natural populations of this species. Sexual reproduction in *H. oligactis* is thought to be a diapausing strategy resulting in resting eggs that can survive winter conditions which the adults can not survive (Reisa, 1973). However, in natural populations sexual, asexual and non-reproductive polyps regularly co-occur at the same time during autumn (Sebestyén et al., 2018). Some of this variation might be explained by genetic differences, as we have previously found different sexual propensity of clonal lineages derived from the same population, when kept under standard conditions in the laboratory (Tökölyi, Ősz, et al. 2017). However, there is clear evidence for phenotypic plasticity in reproductive modes from population genetics of field-collected individuals (Miklós et al., 2019). Based on the results presented in this study, polyp age could be one of the determinate factors of this plasticity, such that younger polyps reproduce asexually, while older ones initiate sexual reproduction when temperature drops during the autumn. In addition to age, external factors such as food availability (Tökölyi et al., 2017) or population density (Bell & Wolfe, 1985) might contribute to variation in reproductive strategies, but the relative role of these factors remains to be elucidated.

The remarkable clonal plasticity observed in *Hydra oligactis* in this study has implications for reproducibility in laboratory studies involving clonal species. Forms of asexual reproduction (e.g., fragmentation, parthenogenesis or budding) are widespread among several model systems (i.e. planarians of the family *Dugesiidae* (Oviedo, Nicolas, Adams, & Levin, 2008), the cladoceran *Daphnia* (Stollewerk, 2010) rotifers (Dahms, Hagiwara, & Lee, 2011), the starlet sea anemone *Nematostella vectensis* (Hand & Uhlinger, 1992) and hydroids of the genus *Hydra* (Galliot, 2012). Clonality is a useful feature in these model species, because individuals of an asexually propagated genotype can be studied in different states and environments. However, these organism are often kept in strain cultures (e.g. planaria: Oviedo et al., 2008, *Daphnia*: Jonczyk & Gilron, 2005, rotifers: Ferreira, Seixas, Coutinho, Fábregas, & Otero, 2011, *Nematostella vectensis:* Hand & Uhlinger, 1992, *Hydra*: Galliot, 2012, implying a mass of asexually propagated animals with varying age. Our study clearly shows that the same environmental effect can induce different resource allocation and reproductive patters at different ages of individual polyps. Therefore, controlling for age in other clonal organisms might reveal similar patterns and certainly enhance the repeatability of studies in other fields.

Overall, our results highlight the role of age in phenotypic plasticity and suggest that the relation between reproductive modes and somatic maintenance changes with age in clonal animals. Using experimental induction of sexual reproduction in *H. oligactis* in different ages, we found that asexual reproduction is favoured early in life with the ability for higher somatic maintenance, while sexual reproduction is preferred later with somatic maintenance disability in the same species. Despite that some clonal species (including *Hydra*) can have exceptional regenerative abilities, low survival rates and negligible or even negative senescence, we found marked age-dependent changes in their reproductive investment and resource allocation decisions. Because we studied these age-dependent differences in sex-induced individuals, our study raises the question whether somatic maintenance changes with age in the asexual stage as well, and if so, how can negligible senescence be maintained with these age-dependent changes. Unfortunately, the plastic modulation of somatic maintenance is understudied in general (Flatt, Amdam, Kirkwood, & Omholt, 2013) and especially in clonal animals, therefore this question remains for future investigations.

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Authors' contribution

F.S., M.M. and J.T. conceived research; F.S., M.M. K.I. and J.T. collected data; F.S. and J.T. analysed data; F.S. and J.T. wrote the manuscript.

Data accessibility

Data for this article is archived in Figshare https://doi.org/10.6084/m9.figshare.8313641.v1 (Sebestyén, Miklós, Iván & Tökölyi, 2019).

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4.3. 3. tanulmány / Study 3: Resource allocation and post-reproductive degeneration in the freshwater cnidarian Hydra oligactis (Pallas, 1766)

Resource allocation and post-reproductive degeneration in the freshwater cnidarian *Hydra oligactis* (Pallas, 1766)

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Abstract

Freshwater hydra are among the few animal groups that show negligible senescence and can maintain high survival and reproduction when kept under stable conditions in the laboratory. Yet, one species of *Hydra* (*H. oligactis*) undergoes a senescence-like process in which polyps degenerate and die after sexual reproduction. The ultimate factors responsible for this phenomenon are unclear. High mortality in reproducing animals could be the consequence of increased allocation of resources to reproduction at the expense of somatic maintenance. This hypothesis predicts that patterns of reproduction and survival are influenced by resource availability. To test this prediction, we investigated survival and reproduction under different levels of food availability in 10 lineages of *H. oligactis* derived from a single Hungarian population. Sexual reproduction was accompanied by reduced survival, but a substantial proportion of animals regenerated after sexual reproduction and continued reproducing asexually. Polyps belonging to different lineages showed differences in their propensity to initiate sexual reproduction, gonad number and survival rate. Food availability significantly affected fecundity (number of eggs or testes produced) with the largest number of gonads being produced by animals kept on a high food regime. On the other hand, survival rate was not affected by the amount of food. These results show that survival is conserved at the expense of reproduction in this population when food is low. It remains a question to be answered why survival is prioritized over reproduction in this population.

Keywords: Cnidaria; food availability; life history; senescence; survival/reproduction trade-offs.

1. Introduction

Cnidarians display one of the simplest but most versatile body organizations within the animal kingdom, characterized by high tissue plasticity and excessive regenerative capabilities (Holstein et al., 2003). Perhaps due to these regenerative capabilities, some members of this phylum display very low rates of senescence (Brock and Strehler, 1963; Martínez, 1998; Boehm et al., 2013; Schaible et al., 2014; Schaible et al., 2015). In a recent study, for instance, Schaible et al. (2015) have shown that *Hydra magnipapillata* and *H. vulgaris* maintain constant rates of fertility and mortality in the laboratory over a period of eight years.

Despite their ability to forego senescence, some *Hydra* species, under specific conditions show increasing rates of age-dependent mortality along with a senescence-like degeneration phenotype. First shown by Brien (1953) and later studied by Yoshida et al. (2006), individuals of *H. oligactis* initiate sexual reproduction when temperature is reduced, after which they die within a few months. Sexual reproduction is followed by a reduction in the number of interstitial stem cells, a decline in the rate of food capture and contractile movements, a decrease in body size and an exponential increase in mortality rate (Yoshida et al., 2006). The reasons why *H. oligactis* undergoes this senescence-like degeneration phenotype are unclear.

According to the disposable soma theory of aging, resources invested into the maintenance of the soma are traded off against investment into reproductive functions (Kirkwood and Rose, 1991). As a consequence, animals with high reproductive investment are expected to show lower levels of self-maintenance and higher levels of aging, a prediction that has received broad support (summarized in Boggs, 2009). Although few systematic studies have been performed on reproductive investment in hydra, studies generally report a higher number of reproductive organs (testes and ovaries) in *H. oligactis* compared to other species (e.g. Schuchert, 2010). This raises the possibility that the post-reproductive degeneration observed in this species might be caused by an increased allocation of resources to reproduction at the expense of self-maintenance functions. Indeed, Reisa (1973) suggested that the "depression" observed in sexually reproducing *H. oligactis* might be the consequence of interstitial stem cells being converted into germ cells instead of nematocysts, which would prevent feeding throughout the sexual cycle.

If post-reproductive degeneration in hydra is caused by the trade-off between survival and reproduction, then the amount of resources available to the animals is expected to influence investments into these functions, with several possible outcomes. First, animals facing a resource shortage might reduce their reproductive investment to increase survival. In some cases experiencing periods of low food availability results in higher levels of stress tolerance and lower rates of aging, a phenomenon termed dietary restriction (e.g. Masoro and Austad, 1996; Partridge et al., 2005; Walker et al., 2005). However, such an effect does not occur in all species (Nakagawa et al., 2012). Within rotifers, for instance, closely related species may show increased or decreased longevity when exposed to the same food restriction treatment (Kirk, 2001). In hydra there is no evidence so far for dietary restriction-mediated increases in self maintenance levels (Bridge et al., 2010; Tökölyi et al., 2016), although species differ in the way in which oxidative stress tolerance is maintained in face of reduced food availability (Tökölyi et al., 2016). Secondly, food shortage might signal increased future mortality risk, resulting in higher investment into reproduction and a reduction in survival, a strategy termed "terminal investment" (Clutton-Brock, 1984; Fischer et al., 2009; McNamara et al., 2009). Such a strategy is seen e.g. in some rotifers (Kirk, 2001; Stelzer, 2001) and birds (Velando et al., 2006) which increase reproductive effort when food availability becomes limited or their immune system is experimentally challenged. Thirdly, in the most simplistic scenario, food shortage may reduce both reproduction and survival at the same time. To test which of these scenarios, if any, occurs in *H. oligactis* undergoing post-reproductive degeneration, we individually followed hydra polyps kept under different levels of food and measured fecundity and survival as two opposing facets of life-history trade-offs. Fecundity was quantified as number of eggs in females, while in males we used the number of testes as a proxy for gamete production. We predicted that fecundity and post-reproductive lifespan would be concomitantly reduced if both functions are valued in the same way, while departures from a parallel reduction would indicate that one of the functions is preserved at the expense of the other, giving rise in the most extreme cases to "dietary restriction" (survival increased and fecundity reduced) or "terminal investment" (fecundity increased and survival reduced) effects.

2. Materials & methods

2.1 Animals and culture conditions

Animals in this study were derived from ten polyps collected in May 2015 from an oxbow lake of the Tisza river near Tiszadorogma, Hungary (47.67N, 20.86E; Fig. 1.). We collected animals that were at least 2 m apart to increase the chance that both males and females are found and to reduce the chance that multiple asexual members of the same genetic clone are collected. Species identity was determined based on morphological traits and investigation of nematocysts (under 400x magnification in a light microscope) based on Schuchert (2010). *H. oligactis* can be distinguished morphologically from other hydra species by the presence of a distinct stalk in the foot region, very long tentacles and asymmetric emergence of tentacles in the buds. We note, however, that species delimition in *Hydra* is far from a resolved issue (see e.g. the recent study of Schwentner and Bosch, 2015), hence the possibility that this population belongs to a distinct branch of the 'oligactis' group cannot, at present, be excluded.

After collection, animals were moved to the laboratory and kept under standardized conditions (20 ºC temperature, 12/12h photoperiod, in a standard hydra medium containing 1.0 mM $CaCl_2$, 0.1 mM $MgCl_2.6H_2O$, 0.03 mM KNO_3, 0.5 mM $NaHCO_3$ and 0.08 mM $MgSO_4$). We propagated these animals asexually by feeding them three times a week with freshly hatched *Artemia* nauplii. This propagation phase lasted for approximately two months, during which the animals were kept in 6-well culture plates in groups of three hydra/well (all three belonging to the same clone).

At the start of the experiments polyps were moved to 24-well plates (1 polyp/well, individuals within a plate belonging to one or two clones). 36 polyps from each original clone were used, resulting in a starting cohort of 360 individuals. To facilitate cleaning, we used special plates with holes on the bottom of the wells forming a sieve (each well had 17 holes with a diameter of 0.7 mm on the bottom). The holes were too small for hydra to pass, but large enough to let out uningested *Artemia* nauplii when the plate was raised from the water. The plates were kept individually in plastic containers with a flat bottom and immersed in hydra medium (wells were approximately half full and polyps were kept in ~1.5 ml medium). A piece of glass (exactly fitting the bottom of the plates) was put below the plates to block experimental animals, detached buds or eggs leaving the wells when the plates were not moved. For cleaning, the plates (with the hydra) were simply taken out of their container and moved to clean containers with fresh medium. To prevent the accumulation of bacteria, we moved hydra polyps to clean plates once every two weeks.

We fed the animals three times a week from a dense *Artemia* slurry using a micropipette, as described previously (Tökölyi et al., 2016). Depending on their treatment, they received either 3, 6, 10 or 20 µl *Artemia* suspension on each occasion (1 µl contained approximately 7-8 nauplii). Food treatments were distributed in plates according to a block design (all four treatments present in every plate). Newly detached buds were counted and removed prior to feeding. After feeding, plates with hydra were moved to containers with fresh medium. Experimental animals were kept under these conditions for two weeks. After that, we lowered the temperature to 7 ºC (temperatures below 12 ºC are used to induce sexual reproduction in *H. oligactis*; Littlefield et al., 1991; Yoshida et al., 2006; Kaliszewicz and Lipińska, 2012; Kaliszewicz, 2015). We also switched photoperiod to a 8/16 h light/dark cycle because there are observations suggesting that a short photoperiod might induce sexual reproduction as well (Reisa, 1973).

To quantify the intensity of sexual reproduction we recorded the number of detached eggs in females and the number of testes in males. Females continually produce and shed eggs during their sexual phase, while males develop several distinct testes along the body column that continuously produce sperm (Reisa, 1973). We counted the number of detached eggs in females three times per week, prior to feeding. Care was taken not to disturb the plates so that detached eggs do not escape through the holes at the bottom of the wells. The number of testes in male polyps was counted once a week under a stereo microscope.

Death was recorded when the animals disintegrated or disappeared between feedings. We were extremely careful to retain experimental animals even if they shrank to a very small size, always checking the plates and the containers in case of a potential death event. Four animals were accidentally lost and we excluded them from all subsequent analyzes.

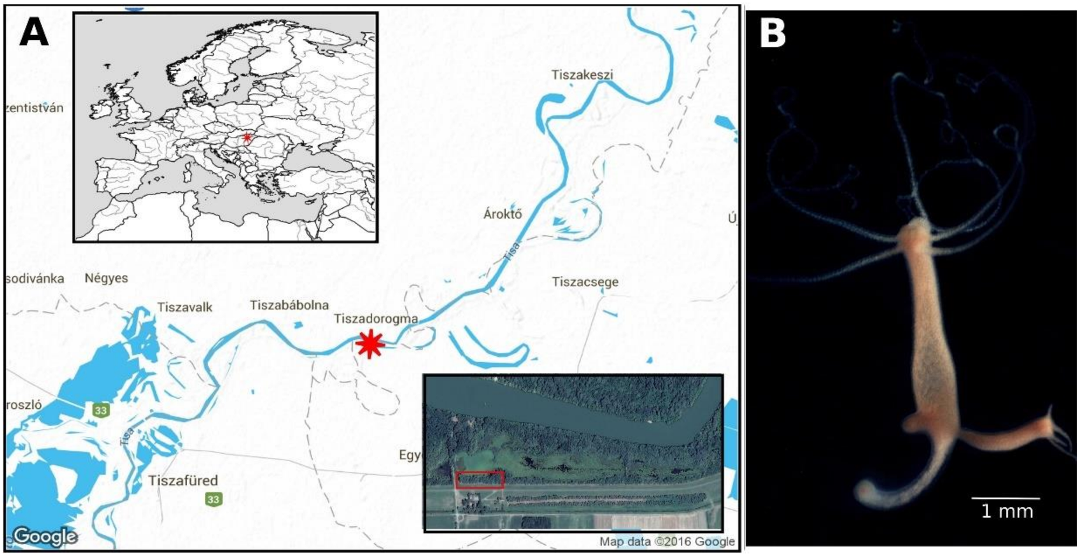

Fig. 1. (A) Map of the Tisza river basin around Tiszadorogma and satellite image showing the site of origin of the population from which founder polyps used in this study were collected (marked by a red star on the map and a red rectangle on the inset). (B) asexual *H. oligactis* polyp from the Tiszadorogma population.

2.2 Statistical analyzes

We analyzed data using Generalized Linear Models (GLMs) or Generalized Linear Mixed Models (GLMMs) implemented in the R Statistical Environment (R Core Team, 2014; using the lme4 R package for GLMMs, Bates et al., 2014). We started by testing whether individual clones differ in their propensity to start sexual reproduction, number of gonads and survival in GLMs with these variables as dependent variables and clone ID as fixed predictor. GLMs with a Poisson distribution were used to analyze fecundity (maximum number of testes/male polyp, total number of eggs produced by a female polyp during the sexual period), while the occurrence of sexual reproduction and survival were analyzed with binomial GLMs (both variables have a binomial outcome: sexual reproduction observed or not and survived or died, respectively). Next, we tested the effect of food treatment on the dependent variables in GLMMs (with either Poisson or binomial distribution). GLMMs were required in this case to take into account that our experimental animals do not represent independent data points (polyps belonging to a given clone are more similar to each other than expected by chance). Hence, in these analyzes clone ID was included as a random variable, in addition to treatment as fixed variable. Lastly, we used GLMMs to test the relationship between fecundity and survival rate. Significance of fixed variables was tested via Likelihood Ratio Tests (LRTs), by comparing models containing the fixed effect to simplified models without it.

3. Results

The pattern of asexual and sexual reproduction and survival changed over the course of the experiment as shown on Fig. 2. Lowering the temperature inhibited budding for approximately two weeks, then animals produced again buds for two weeks, which was followed by initiation of sexual reproduction. Of the ten clones five were found to be males and five to be females. The number of days elapsed from the start of the temperature manipulation to the initiation of sexual reproduction was 38 ± 3.91 days in males and 47 ± 5.65 days in females (mean ± SD presented in both cases). Survival rate started to decrease after initiating sexual reproduction, but a substantial proportion of animals survived and started budding again. We stopped the experiment after 150 days, when most of the animals were either dead (N=109, 31% of the initial cohort) or regenerated after sexual reproduction and produced at least one bud (N=176; 49%). The remainder either did not initiate sexual reproduction until the end of the study (N=33, 9%) or were in an intermediate state (i.e. survived until Day 150 but did not produce any buds following sexual reproduction; N=38, 11%). We scored these intermediate animals as survivors (even though some of them might have died later without regenerating) because they fulfill the criterion of surviving until Day 150. We found no bias in the distribution of these animals according to food treatment (N=8, 6, 12, 12 animals out of N=55, 64, 64, 64 surviving animals in the 3, 6, 10, 20 µl Artemia groups, respectively; Fisher's Exact Test, p=0.539).

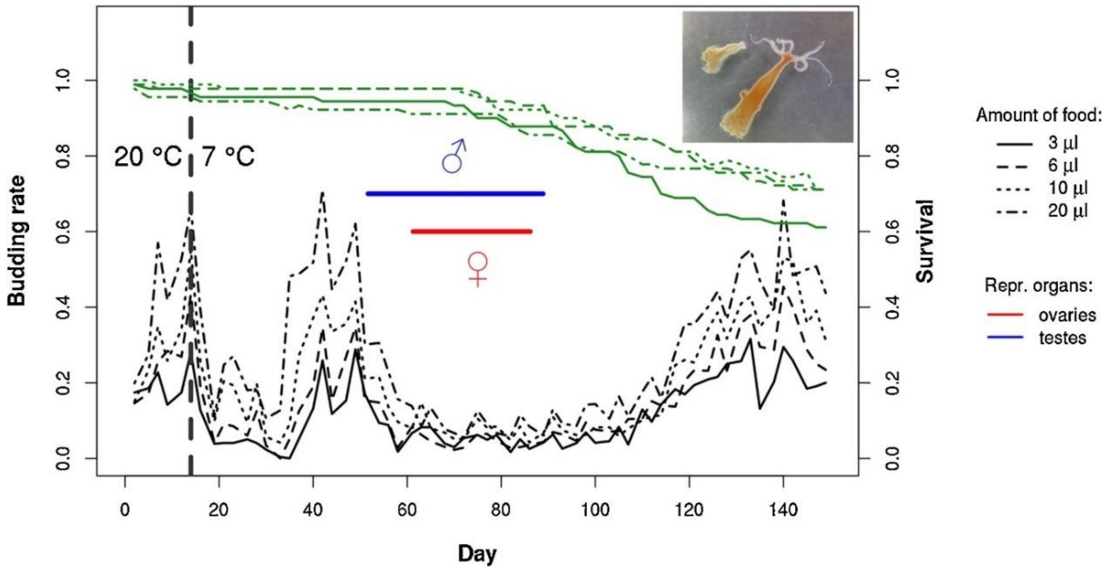

Fig. 2. Temporal patterns of asexual and sexual reproduction and survival rate of *H. oligactis* polyps kept on four different food regimes. Black lines show the budding rate (number of buds/day), green lines show the proportion of the initial cohort still surviving. The mean days for the presence of gonads in males and females are indicated by blue and red lines, respectively. The inset shows two polyps towards the end of the experiment in different stages of degeneration; the individual on the right regenerated almost completely.

Individual clones differed significantly in their propensity to initiate sexual reproduction (binomial GLM, deviance=163.62, df=9, p<0.001; Fig. 3A) and in the probability of surviving to the end of experiment (binomial GLM, deviance=75.325, df=9, p<0.001; Fig. 3B). The number of testes produced by males were marginally significantly different between clones (Poisson GLM, deviance=9.112, df=4, p=0.058; Fig. 3C). The total number of detached eggs significantly differed between female clones (Poisson GLM, deviance=35.952, df=4, p<0.001; Fig. 3D).

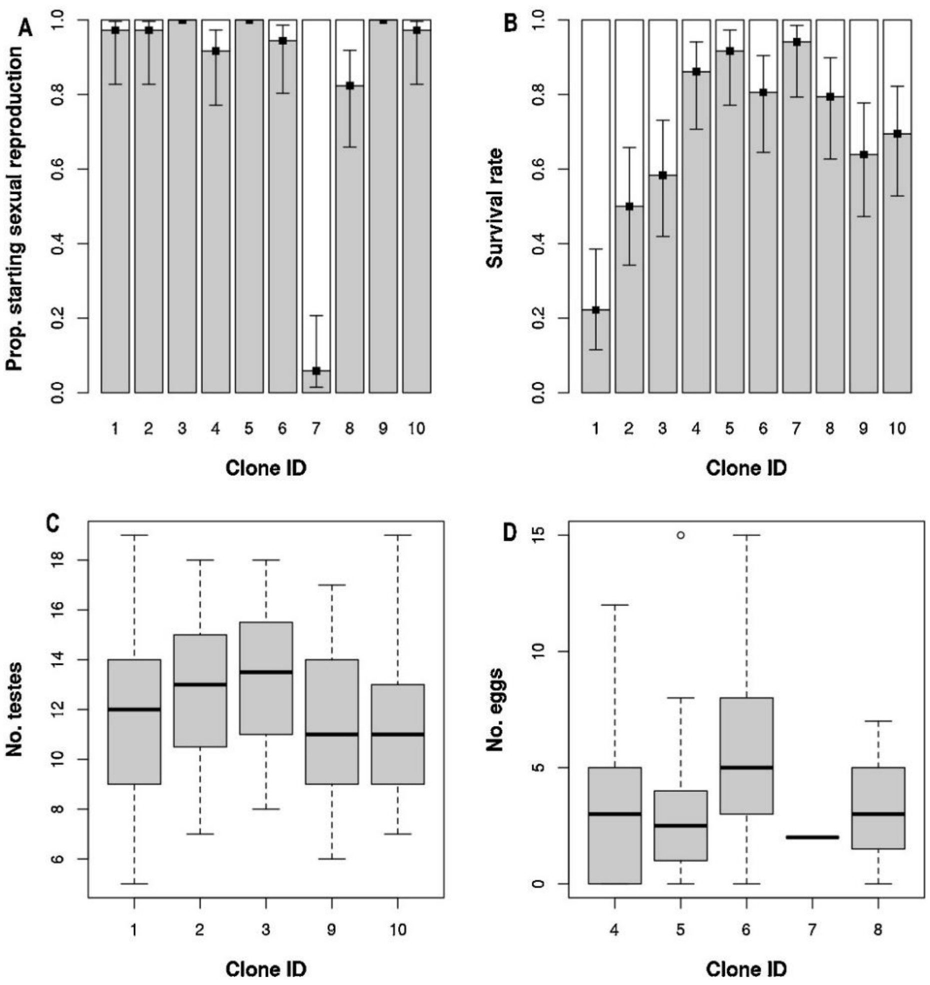

Fig. 3. Differences between 10 *H. oligactis* clones derived from the same population in (A) their propensity to initiate sexual reproduction, (B) survival rate, (C) maximum number of testes (only male clones shown) and (D) total number of detached eggs (only female clones shown). Error bars in panels A and B represent 95% confidence intervals obtained from binomial GLMs.

Food treatment had no effect on the occurrence of sexual reproduction (binomial GLMM, $\chi^2=2.829$, df=3, p=0.419), but it had a significant effect on the number of testes in males (Poisson GLMM, $\chi^2=69.454$, df=3, p<0.001; Fig 4A) and the number of eggs in females (Poisson GLMM, $\chi^2=54.015$, df=3, p<0.001; Fig. 4B). Survival probability was not affected by food treatment (binomial GLMM, $\chi^2=3.854$, p=0.278; Fig 4C). Furthermore, survival probability was not related to fecundity neither in males (maximum number of testes, binomial GLMM, $\chi2=1.800$, p=0.180), nor in females (total no. eggs, binomial GLMM, $\chi2=0.537$, p=0.464). Lastly, excluding polyps that survived until the end of the experiment but did not regenerate following sexual reproduction (N=38 animals) had no qualitative effect on our results (not shown).

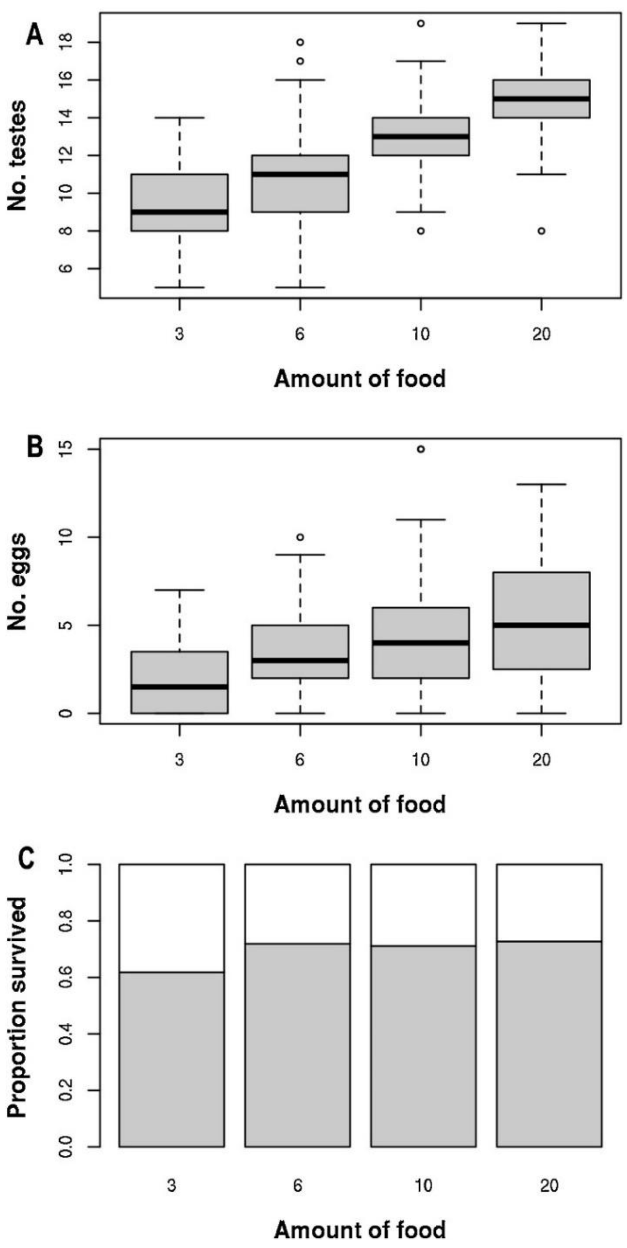

Fig. 4. Effects of food treatment on (A) the maximum number of testes produced by males, (B) the number of eggs produced by females, and (C) the survival rate.

4. Discussion

In this study we investigated the fecundity/survival trade-off under different food levels in male and female *H. oligactis* undergoing sexual reproduction. We found that polyps initiated gonadogenesis irrespective of food availability, but the number of gonads (testes or detached eggs) increased with food. Following sexual reproduction there was high mortality (>30% of the initial cohort died during the course of the experiment), but survival rate was not related to the amount of food.

Sexual reproduction in *H. oligactis* is generally thought to be an escape strategy that enables hydra to produce resting eggs before the onset of the winter (Reisa, 1973; Kaliszewicz and Lipińska, 2012). According to this hypothesis, a sudden drop in temperature signals the beginning of a period with high mortality due to low temperatures and/or starvation, and animals are selected to quickly develop reproductive organs and produce eggs that are able to survive these adverse conditions. Our observations suggest that this hypothesis cannot fully explain the occurrence of sexual reproduction in this population. First, lowering the temperature inhibited asexual reproduction only temporarily and the sexual phase started after a period of asexual reproduction in the cold. This suggests that temperature is a cue rather than a physiological constraint that shapes the type of reproduction (Reisa, 1973); the change in temperature could serve to indicate the time when sexual reproduction is ecologically most favoured. This could be in the autumn, as supported by field observations of sexual reproduction in this species in Switzerland (Ribi et al., 1985) and Michigan, USA (Miller, 1936), but is probably later in our Hungarian population (based on observations in this study). Secondly, similar to the study of Kaliszewicz and Lipińska (2012), the amount of food in our experiment had no influence on the probability of initiating sexual reproduction. Together, these observations suggest that initiation of sexual reproduction in *H. oligactis* is not related to the amount of food, although other aspects of food, such as its predictability are known to influence hydra life history traits (Schaible et al., 2011; Rosa et al., 2016) and might be involved in this case as well.

The delayed initiation of sexual reproduction in animals exposed to cold and the high post-reproductive survival rate of *H. oligactis* polyps from our population is surprising. In most published accounts of sexual reproduction in this species (e.g. Brien, 1953; Yoshida et al., 2006; Kaliszewicz and Lipińska, 2012; Kaliszewicz, 2015), exposure to cold temperatures inhibits budding and initiates gametogenesis within 2-3 weeks, after which polyps face an exponential increase in mortality rate (Brien, 1953; Yoshida et al., 2006). The experimental animals in this study experienced progressive degeneration and a decrease in body size after sexual reproduction very similar to that reported by Yoshida et al. (2006); however, a substantial proportion survived, regenerated and resumed asexual reproduction. Although these differences might be at least partly caused by differences in experimental setup (e.g. both temperature and photoperiod differed between the studies), intraspecific differences in post-reproductive survival are likely to exist, as Tomczyk et al. (2015) recently reported a strain of *H. oligactis* that does not experience increased post-reproductive mortality. The physiological mechanisms responsible for this variation are unclear. Post-reproductive degeneration in *H. oligactis* is generally thought to be the consequence of stem cell depletion ("gametic crisis"; Brien, 1953; Reisa, 1973; Tardent, 1974; Yoshida et al., 2006; Bosch, 2008). Sexual reproduction and germ cell development in hydra depends on stem cell activity (Littlefield, 1985; Littlefield, 1991), hence, increased commitment of stem cells to germ cell production is likely to reduce the differentiation of multipotent stem cells into somatic cells necessary for survival. The study of Yoshida et al. (2006) illustrates this pattern well, since they found a marked increase in germ cell numbers and a drastic decrease in epithelial cells, interstitial stem cells and somatic derivatives (nematocytes, nerve cells, and gland cells) during sexual development. Therefore, an obvious explanation for the high post-reproductive survival of the hydra population studied by us could be that in animals surviving sexual reproduction fewer stem cells are converted into germ cells and a sufficiently large subpopulation of stem cells remains to regenerate the whole animal. This is a prediction that should be tested in the future.

From an ultimate (evolutionary) perspective, differences between populations might be related to environmental conditions (e.g. the risk of freezing) in the natural habitats of these populations/strains. For example, a quick and predictable onset of winter would select for a rapid induction of gametogenesis upon cold exposure.Other factors are likely to be involved as well, because we found that within a single population several different life-history strategies can coexist. Even within this admittedly very limited sample of 10 clones we found individuals that initiated sexual reproduction with high probability while others continued budding during the whole period (results not shown). Furthermore, some lineages survived with high probability while in others survival rate was less than 25%. These differences could represent alternative life history tactics within the population (e.g. local adaptations to differences in microhabitat) or a consequence of temporally varying selection on life history traits (e.g. Mojica et al., 2012). It could also be the consequence of a high rate of influx of genetically distinct individuals into the lake from which our founder polyps were derived. This latter hypothesis is highly plausible, given that the lake is very close to the Tisza river, it is directly connected to it and periodically flooded by the river (the Tisza is a major river of the region with a large drainage area and is a known habitat of *H. oligactis*; Gelei, 1930). Interestingly, we also found that polyps belonging to a single clone and kept under identical conditions exhibited variation in life history traits (e.g. some polyps did not initiate sexual reproduction, even though most individuals belonging to the same clones did), which could be a form of bet-hedging (diversifying reproductive strategies to increase the chance that some of them will be successful in a highly unpredictable environment; e.g. Olofsson et al., 2009).

Experimental manipulation of food availability significantly affected fecundity, but had no effect on survival rate. Hence, at least over the range of food levels used in this experiment, sexually reproducing *H. oligactis* polyps preserved survival at the expense of reproduction. This pattern is not consistent with a "dietary restriction" or "terminal investment" effect, but neither with a model in which survival and reproduction are equally dependent on food availability. On the other hand, a reduction in the rate of reproduction and maintenance of somatic functions in face of challenging conditions has been observed e.g. in reef corals (Leuzinger et al., 2011), and is generally predicted to occur in long-lived, iteroparous animals that are expected to favour survival over current reproduction (e.g. Gaillard et al., 2000; Therrien et al., 2008; Kitaysky et al., 2010; Monteith et al., 2013). This is somewhat surprising, since *H. oligactis* is a species with a relatively fast life history (it is characterized by high rate of asexual reproduction and low tolerance against oxidative stress; Tökölyi et al., 2016). Since most of the animals from this population did not die after sexual reproduction, investing more resources into survival might enable them to survive and start asexual reproduction again following a sexual cycle (i.e. they might be iteroparous). Based on this hypothesis, the relationship between food availability and survival rate might be quite different in populations/strains that do not survive after sexual reproduction (i.e. semelparous populations). This is a prediction that could be tested in the future.

Our results do not provide unequivocal support for the hypothesis that post-reproductive degeneration is a consequence of reduced allocation of resources to somatic maintenance in sexually reproducing hydra. First, survival rate was not influenced by food availability (although, as discussed above, this could be a consequence of strategic investment of resources into survival at the expense of reproduction). Second, we found no relationship between fecundity and survival rate in individual polyps, which is a key prediction of the hypothesis that these two are traded off against each other. We must emphasize, however, that variation in reproductive investment within our sample might not be enough to detect such a relationship, since the individuals used in this analysis were derived from 10 clones and variation within clones might be too small. Further studies should (i) investigate the relationship between fecundity and survival rate on a much wider sample of hydra populations kept under identical conditions and (ii) attempt to manipulate reproductive investment independently from food (e.g. by manipulating mate availability or population density as ultimate factors, or potential physiological regulators as proximate factors) to unequivocally answer this question.

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