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Effects of different environmental factors on life history of hydrozoan *Eleutheria dichotoma*

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1 Introduction

1.1 General introduction

During their life, organisms experience variation in environment that can disturb their internal homeostasis. Maintaining particular components of organism's internal environment constant is often a big challenge. There are two stressors: abiotic (e.g. temperature, climatic factors, chemical components) and biotic (caused by living organisms, e.g. competition). Many organisms can response to stress by changing their phenotypes. Production of different phenotypes by one genotype in different environments is known as phenotypic plasticity. It can be manifested in morphological, behavioural, physiological, or life history traits. An outcome of phenotypic plasticity are compensatory reactions of an organism, which can be discrete or continuous (rapid or gradual) and reversible or irreversible (Rilov and Crooks 2008, p. 179). An example of discrete irreversible phenotypic response is the temperature induced sex determination in reptiles. Discrete but reversible changes include e.g. predator-induced diurnal vertical migration in zooplankton (Bollens and Frost 1991). Continuous reversible phenotypic responses are evident in many physiological and morphological traits e.g. physiological acclimation to seasonal temperature change in ectotherms. Finally, continuous but irreversible phenotypic changes occur in many life history characteristics, e.g. altering timing of metamorphosis in many invertebrates in response to various environmental cues e.g. temperature (Rilov and Crooks 2008, p. 179). Changes that are inherited and passed to further generations are known as evolutionary adaptations (Garland and Kelly 2006).

Another strategy of a response to variation in environment is bet-hedging. It occurs when organisms have decreased fitness in their typical environment, but may have increased fitness in unfavourable environments. For example, production of phenotypes with lower average reproductive success, but with increased variability in different life history strategies

(e.g., survival) can be beneficial in variable environment, where some conditions are favourable for some phenotypes, but harmful for others (Starrfelt and Kokko 2012; Botero et al. 2015).

The average contribution of a genotype/phenotype to the gene pool of the next generations is called (Darwinian) fitness. Phenotypes with higher fitness are more likely to dominate the population. For many ecological scenarios fitness is simply measured as expected number of offspring produced during the whole life (lifetime reproductive success) (Kozłowski 1993; Dańko et al. 2018b). An organism is typically limited by available resources and physiological constraints and thus is forced to trade-off. It means that a beneficial change in one trait is detrimental for another trait – both traits cannot be simultaneously "maximized" by evolution. In the context of optimal resource allocation theory, the limited amount of resources has to be partitioned among different non-reproductive and reproductive activities in a way that maximizes fitness (Gadgil and Bossert 1970; Kozłowski 1992). In organisms which have differentiated soma and germ cell lines the benefits from investment in reproduction are almost immediate, while benefits from investment in soma are expected only in future. Under unavoidable mortality risk such investments might be never paid back (Cichoń 1997). In other words, investment in own body is reasonable as long as it can increase future reproductive success. For example, annual organisms are limited by a length of the growing season. A typical annual organism invests resources in growth at beginning of its life and then it completely or partially switches from growth to reproduction (Kozłowski 1992). Allocation patterns are strongly dependent on mortality risk during the season. Conversely, a typical perennial organism should allocate some part of available energy to growth and maintenance each season (Kozłowski 1992). The additional investment in vegetative growth can be repaid if organism has a high probability of survival to the next season. The optimal allocation theory explains compartment of resources in organisms with complex life cycle, where subsequent life forms differ in reproduction and survival strategies. Furthermore, the theory is not only limited to

organisms in which soma and germ line can be distinguished, but can also be applied to much simpler organism such as *Hydra sp.* (Dańko et al. 2015).

The environmental stress factors can affect patterns of resource allocation of an organism. Coping with a stress may involve various expensive physiological or behavioural mechanisms such as increased tissue repairs and maintenance, osmoregulation, migration, etc. These expenses limits resources for other aspects of life. For example increased investment in osmoregulation could lead to decreased growth, reproduction and/or tissue repairs. Decreased allocation to maintenance and repairs can in turn lead to faster senescence of the soma in an organism in which the distinction to soma and germ line can be made; where senescence is defined as a decrease in the reproduction rate and an increase in the mortality rate with age (Dańko et al. 2015; Schaible et al. 2015). An environmental stressor can also act less directly on allocation patterns. For example, in environments characterized by high extrinsic mortality the investment in prolonged longevity seems to not be an optimal decision (Williams 1957; Kirkwood 1977), and a shift of the resources from maintenance and tissue repairs to reproduction is expected, which leads to faster aging (Cichoń 1997; Dańko et al. 2018b).

Although aging is mainly related to soma, in organisms without the soma-germ distinction, accumulated damage can be easily passed to the next generations. This process can even accelerate in organisms reproducing asexually via mechanisms known as Muller's Ratchet (Felsenstein 1974). Sexual reproduction has clear benefits for an organism, because it gives a chance to escape the Ratched by gene recombination. This reproductive strategy leads to the production of more robust life forms that can overcome or escape unfavorable conditions (e.g. Kleiven et al. 1992). From the other hand, asexual reproduction is a fast and effective way of propagation of a clone and might enable a rapid colonization of new environments (Schlesinger et al. 2010). It is typically less costly mechanism than sexual reproduction in terms of finding a partner or producing gametes.

One of the ecosystems characterized by high environmental variability is marine ecosystem. Marine ecosystems differ from freshwater ecosystems by a high salt content. They cover more than 70% of the surface of the Earth and include nearshore systems (e.g. coasts, estuaries or rocky intertidal pools) and offshore systems (e.g. surface ocean, pelagic ocean). The main factors that shape life history response in marine ecosystems are food availability, temperature and salinity. For example, an annual cycle of phytoplankton is the main biological trigger of seasonal variation, regulating reproduction of marine organisms at every level of the trophic chains (Bavestrello et al. 2006; Sommer et al. 2012). The phytoplankton seasonal growth pattern is mainly determined by physical factors such as light and stratification, as well as the limitation of nutrients (Sommer et al. 2012). The seasonal values of light intensity contrast with concentrations of nutrients. While light intensity is the highest in summer and the lowest in winter, the concentration of available (dissolved) nutrients is climate-specific. For example in Mediterranean Sea, the highest concentration of nutrients is observed during winter and early spring, which results in the rapid increase of algae communities (Coma et al. 2000). Conversely, in Baltic Sea the peaks of algae communities occur in summer (Schubert et al. 2017). The dynamic of algal populations is driven by limiting factors, different for planktonic and benthic communities (Sommer et al. 2012). During the spring in Mediterranean Sea, the highest production of algae occurs in the shallow waters, whereas in summer it occurs in the deep water parts (Coma et al. 2000).

Variation of temperature is the most pronounced in environments with temperate climate. Decreasing temperatures and deterioration of food conditions indicate the end of the reproductive season, which can be a trigger for entering a resting stage or starting sexual reproduction in many organisms (e.g. Kleiven et al. 1992). Coping with changes in temperature requires specific adaptations. There are two main types of response that have evolved in the tree of life: endothermy and ectothermy. The endotherms maintain their body at metabolically

favorable temperatures using their endogenous source of heat which is their routine metabolism (Johnston and Bennett 2008). The ectotherms have low possibilities of controlling their body temperature and rely mainly on environmental heat sources. All invertebrates and most vertebrates belong to ectotherms. Their growth and development strongly depends on the temperature. It was observed that most of the ectotherms grow faster in the high temperature, however the maximum body sizes are lower than in low temperature. This observation has been called Temperature-Size Rule (TSR) (Atkinson 1994; Ghosh et al. 2013). Response of the organism to temperature during its development is a form of phenotypic plasticity, because one genotype may express different phenotypes depending on the temperature of its environment. It is not clear what kind of general physiological mechanism might regulate response to temperature. It is also not clear if phenotypic response to temperature is a product of physiological constraint (Walters and Hassall 2006) or combination of multiple factors (Angilletta et al. 2004).

In marine environments the temperature often interacts with salinity, which is an another key factor affecting the physiological and ecological responses of marine organisms (Kinne 1970; Remane and Schlieper 1972; Holst and Jarms 2010; Telesh and Khlebovich 2010). Mechanism for coping with osmotic stress on the cellular level is cell volume regulation (Hoffmann and Pedersen 2011) and is often associated with energetic costs (Sokolova et al. 2012). The physiological mechanisms of cell volume regulation involve osmoregulation (ionic regulation of intra- and extracellular fluids through ion channels, ion exchange proteins or primary ion pumps) and osmotic adaptation (through intracellular concentration of osmotically active substances e.g. free amino acids (Mayfield and Gates 2007; Schubert et al. 2017).

Many marine invertebrates do not perform osmotic regulation of their extracellular fluid and rely solely on intracellular regulation of cells' volume, which should have low energetic costs. So called, osmoconformers are normally restricted to marine waters and adopt to low

range of salinity. Generally, their energy requirements measured in respiration rate are low at decreased salinity, and high at increased salinity, probably due to increased production of osmotically active substances (Rivera-Ingraham and Lignot 2017). Osmoregulation, which is performed by many marine invertebrates and all marine vertebrates enables maintenance of a difference in ionic concentration inside and outside the cells at appropriate physiological levels. It is energetically costly process in terms of respiration and aerobic metabolism (Gaudy et al. 2000; Rivera-Ingraham et al. 2016). Osmoregulation can compromise many aspects of life including immune defence (Birrer et al. 2012), growth (Pechenik et al. 2000; Sampaio and Bianchini 2002), and reproduction(Pechenik et al. 2000; Yin and Zhao 2008).

One of the most important biotic environmental factor that can affect the fitness-related traits of an individual is population density. Increased population density can cause resource depletion, increased stress from intra- and interspecific competition, and increased accumulation of harmful metabolites. Density-related factors can activate various direct and indirect mechanisms of somatic deterioration, which may in turn lead to decline in organism's performance with age. In particular, population density can trigger a change in resource allocations strategies, and may affect life history traits such as growth rate, age at maturity, adult body size, and lifespan (Kozłowski 1992; Dańko et al. 2018b). There are two classical models describing strategies of investment in growth and reproduction in response to resource-limited environments: r- and K-selection models. The theory was proposed by Wilson and MacArthur (1967) and was applied by Pianka (1970) to the evolution of life histories. For example, r-selection occurs in variable and unpredictable environments with density-independent mortality. In such conditions organisms generally remain in small populations. Organism that apply r-selection strategy are characterized by rapid growth of a population, a high degree of dispersal, high levels of reproductive effort, a tendency to mature early, a small average body size and closely spaced generations. K-selection, in contrast, occurs in fairly

constant and predictable environments with density-dependent mortality. It should occur among organisms that are near carrying capacity; that live in resource-limited, competitive environments; and that are subject to strong density effects. Species applying K-strategy should have slow development, delayed reproduction, larger body size and long lifespans. In the modern view on K-selection, organisms employ a variety of strategies, some of which would fit better in the r-selection than in the K-selection model (Stearns 1992; Reznick et al. 2002; Kozłowski 2006). Nonetheless, it seems clear that organisms with initially low levels of regulation via density dependence should reproduce more rapidly, as the rapid propagation of a genotype is the most effective strategy for increasing fitness (which is analogous to the r-strategist concept). On the other hand, when density levels are high, organisms should invest more resources in growth and tissue maintenance in order to maximize their quality, and thus the future reproduction in a competitive environment (K-strategists concept; (Pianka 1970; Stearns 1992; Kozłowski 2006; Dańko et al. 2017a)).

In natural habitats, resources are typically unequally partitioned among individuals, and this inequality increases when resources become scarce; i.e., due to increased population density. Łomnicki (1978) showed that unequal resource partitioning is a source of variation among individuals and may lead to competition for resources. Intraspecific competition can be partially or fully avoided by complex life-cycles (Łomnicki 1988), in which the resources used at different life stages do not overlap. Complex life cycle is a strategy to survive in environment, which may not be suitable during the whole year. Developing resting life stages (e.g., menonts, diapausing eggs, and cysts) and mechanisms of brood protection (resistant egg membranes, gelatinous egg covers) increases survival during adverse conditions (Kinne 1970; Bailey et al. 2004; Ma and Purcell 2005) and is advantageous in ephemeral, short-term, and seasonally variable environments.

The majority of marine invertebrates have complex life-cycles, such as separate benthic and planktonic life stages (Eckman 1996). The life-cycle of many cnidarians is characterized by the occurrence of structurally and functionally different polyp and medusa life forms. Medusae generations are produced by polyps, but these two life forms can occur simultaneously over the course of a year (Ceh et al. 2015; Morandini et al. 2016). Another taxonomic feature of cnidarians is the great diversity of the reproductive strategies these animals employ, with asexual reproduction playing an important role (Fautin 2002).

1.2 Model organism

Hydrozoan *Eleutheria dichotoma* Quatrefages, 1842 (Cnidaria: Hydrozoa: Cladonematidae) belongs to group of budding hydromedusae, which do not swim, but creep on algal substrate and produce clonal medusae by asexual budding (Hauenschild 1956; Hirano et al. 2000; Mills 2007; Kawamura and Kubota 2008). *E. dichotoma* has two main life forms of free-living, crawling medusae and benthic, colonial polyps (hydroid colonies) (**Fig.1.1; Fig. 1.2**).

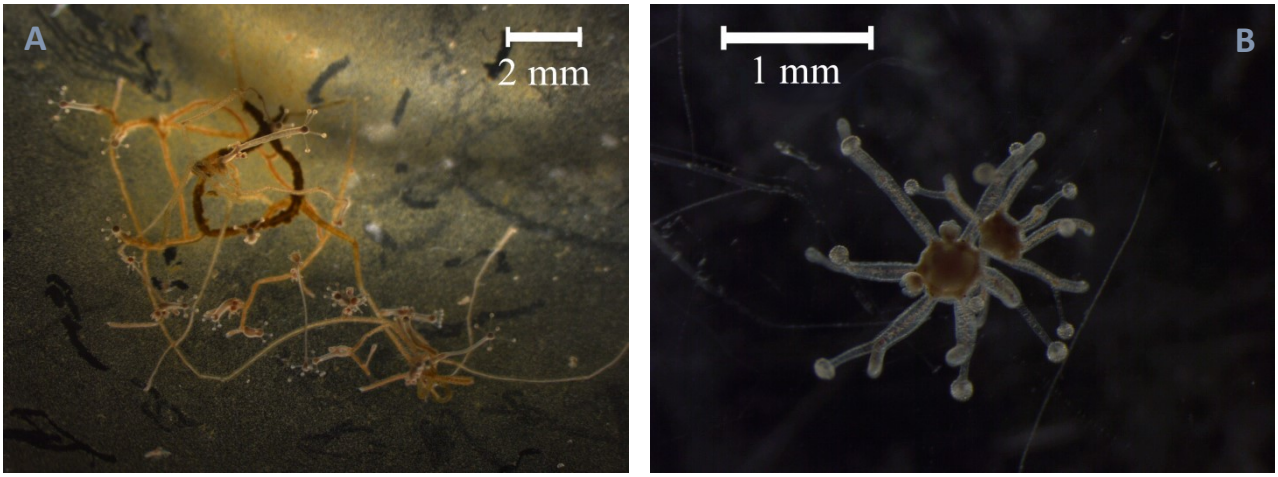

Figure 1.1 Colonial hydrozoan Eleutheria dichotoma. ***A. Hydroid colony*** *grows by producing stolon (hydrorhiza) and its branches (hydrocladia) bearing one or more polyps (hydranths). Each hydranth has gonophores developing into medusa buds.* ***B. Medusa*** *reproduces asexually by producing medusa buds, which develop on external rim of its umbrella, or sexually by producing planula larvae.*

The medusae buds are produced in the basal region of each hydroid, on a gonostyl (**Fig. 1.3**), and are released at their advanced stage of development. Medusae are small (less than 0,5 mm bell diameter), non-swimming and have five bifurcated tentacles (**Fig. 1.4**). Young medusae start to reproduce asexually by budding consecutive generations of medusae. The buds develop on aboral side of medusae on external rim of umbrella. After three to four weeks medusae mature sexually and start releasing planula larvae (Hauenschild 1956) (**Fig. 1.2** and supplementary materials **Fig. A.1**).

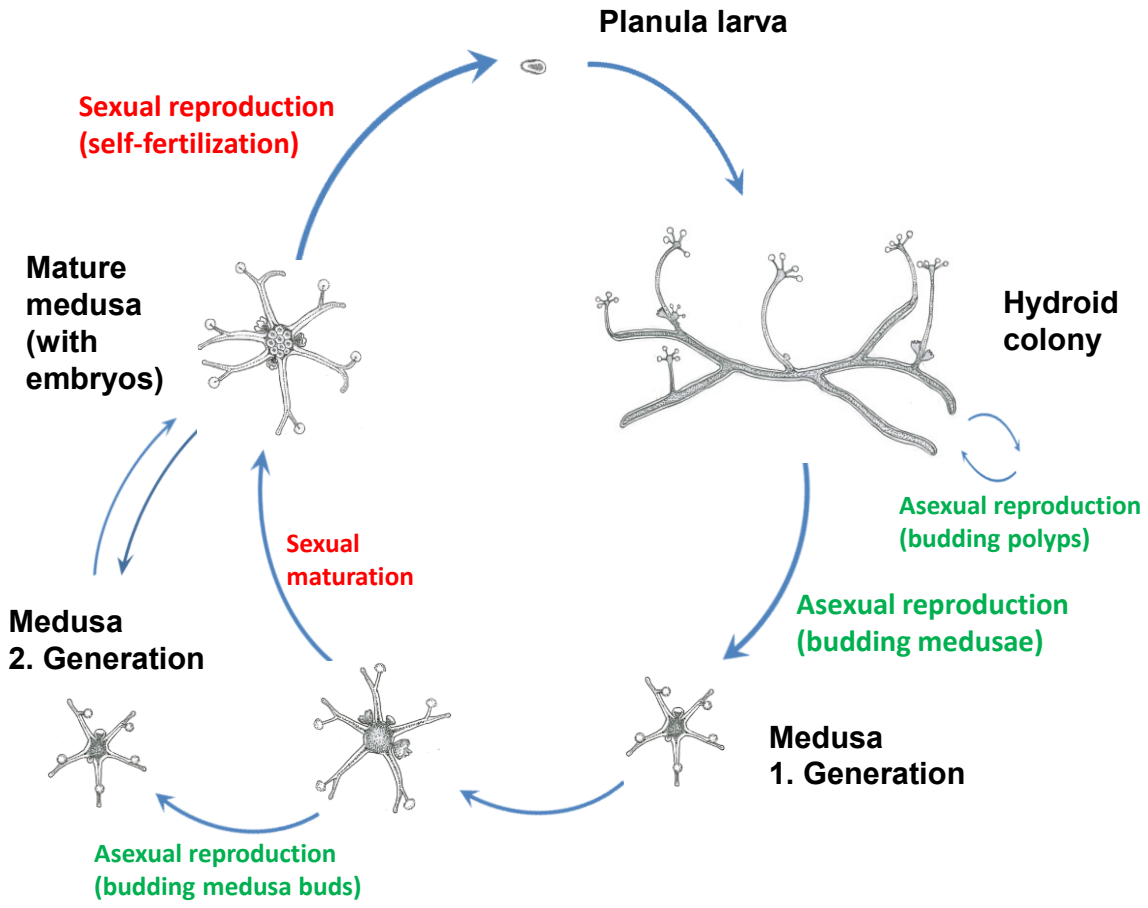

Figure 1.2 Life cycle of Eleutheria dichotoma

Medusae are simultaneous hermaphrodites. Eggs and sperm are released and fertilized within a brood pouch (self-fertilization). Embryos develop initially in a brood pouch placed on an aboral

side of umbrella and they are released as planula larvae through tearing of umbrella (Hauenschild 1956; Schierwater and Hadrys 1998). After sexual maturity, asexual reproduction may cease or may continue, but on the lower level, which is dependent mainly on food availability (Hadrys et al. 1990). Medusae are seasonal, probably dying with the drop of the temperature announcing winter (Schierwater and Hauenschild 1990). Mature medusae tend to favor sexual over asexual reproduction in response to different environmental stressors: e.g., toxicity (cadmium), low temperature, and food scarcity (Schierwater and Hadrys 1998).

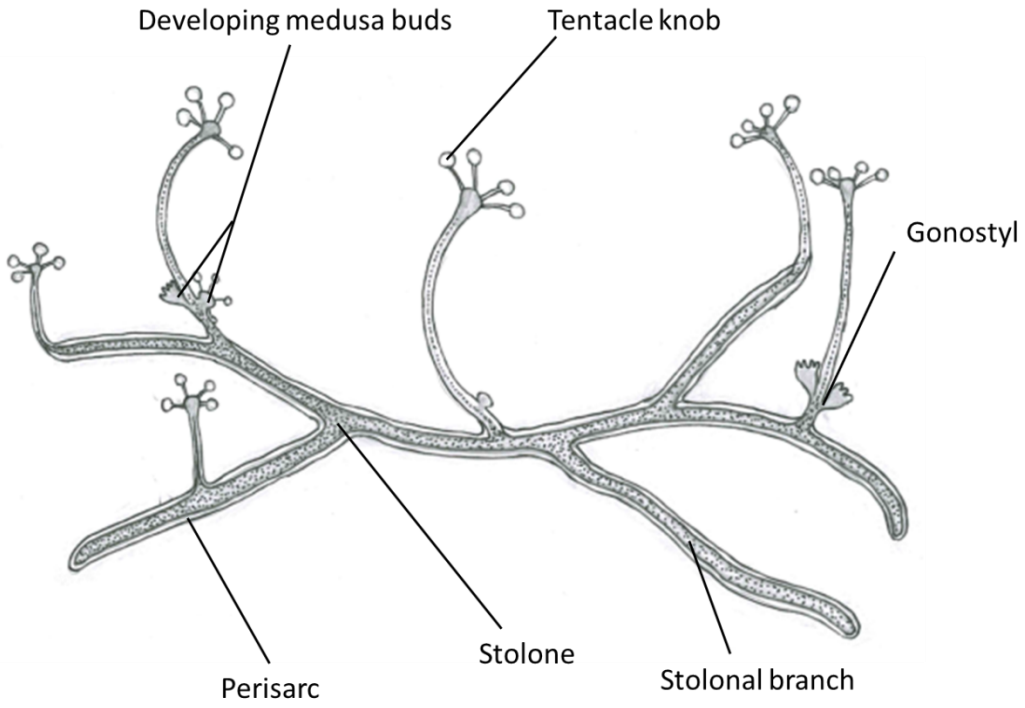

Figure 1.3 Young hydroid colony of Eleutheria dichotoma. Polyps (hydranths) are connected to each other by stolon (hydrorhizae) and have common gastric cavity. The stolonal tissue (coenosarc) is covered by a thin layer of exoskeleton (perisarc), which is transparent by young colonies, and turns dark and thick by older colonies (see Fig. A8 in supplementary materials). The colony grows on the tips of the stolon (hydrorhizae) and on the tips of stolonal braches (hydrocladia). Medusae buds develop at the base of each polyp, from a tissue mass termed entocodon and remain attached to polyp through gonostyl.

Free-swimming larvae use their own energy reserves (Collins 2002; Marshall and Bolton 2007), which allows them to survive few days. Planula larvae, which succeed in settlement, metamorphose into primary polyp, starting new hydroid colony. Due to the high mortality risk

of medusae and larvae, the success of metamorphosis into polyp stage can be very low and difficult to estimate (Purcell 2017).

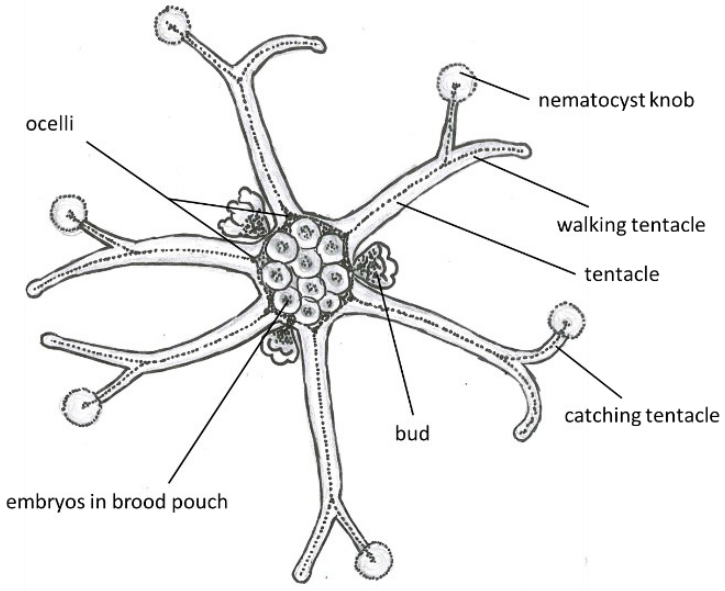

Figure 1.4 Mature medusa of Eleutheria dichotoma viewed from the aboral side, with larvae developing in a brood pouch. About 25-fold magnification.

The polyps (hydranths) are connected to the other polyps by hydrorhiza (system of stolons) and have a common gastric cavity, which facilitates transport of nutrients between the hydranths and hydrorhiza (**Fig. 1.3**). Stolons are creeping, branched, and covered with perisarc (the structure mainly composed of chitin). Perisarc serves for attachment to the substrate, protection and support (Bouillon et al. 2004). The stolons in colonial hydroids are built of tubular coenosarc, which is covered with a thin layer of exoskeleton (perisarc). Perisarc is composed of an outer layer of polysacharyds (including chitin) and an inner layer of structural proteins (Gili and Hughes 1995) and serves as the protection of the tissues and attachment to the substrate (Bouillon et al. 2004). The hydroid colony grows by elongation of the tips of hydrorhiza, by elongation of hydrocladia (stolonal branches) (Gili and Hughes 1995) and by budding new hydranths. Polyps are perennial an can endure low temperatures during winter.

They are source of medusae in early spring when temperatures increase. Polyps are hardly found in nature (Schuchert 2009), but their circannual cycle of activity in the wild presumably reflects periods of occurrence of medusae (Schierwater and Hauenschild 1990).

Eleutheria dichotoma occurs in many locations along the Atlantic coast of Europe including Norway (average salinity of 34.5), the Mediterranean (average salinity of 38), and the Black Sea (salinity of 18); but is probably absent from the Baltic Sea (average salinity of 10) (Belkin et al. 1998; Madsen and Höjerslev 2009; Schuchert 2009; Borghini et al. 2014). *E. dichotoma* has also been found, as a non-indigenous species, along the east coast of Australia (Fraser et al. 2006). The species inhabits coastal intertidal water zones and shallow depths, and is most frequently found in tidepools. Such habitats can be exposed to considerable variation in salinity, which may be determined by the rate of seawater flushing, seasonal changes (freezing, evaporation, rainfall), and the distance of the pools from the seashore (Metaxas and Scheibling 1993). *Eleutheria dichotoma* might theoretically predominate in tidepools, as the relatively high water temperatures in these reservoirs may facilitate asexual reproduction (Ma and Purcell 2005; Kawamura and Kubota 2008). Surprisingly, in their natural habitats in the Mediterranean Sea, medusae have always been observed in very low densities (Schierwater and Hauenschild 1990). This finding suggests that populations of *E. dichotoma*, in its natural environments is strongly regulated by different abiotic and biotic environmental factors.

1.3 Research hypotheses

The goal of my study was to investigate the effects of the two abiotic environmental factors (temperature and salinity) and one biotic factor (population density) on life cycle of hydrozoan *Eleutheria dichotoma*. I expected to find that coping with environmental stress should affect resource allocation patterns determining life-history strategies of growth, survival and reproduction. Particularly, I expected different responses to stress by medusae and hydroid colonies. These two life forms occupy different functional and ecological niches: hydroid

colony is reservoir of polyps and medusae, while medusae are a source of medusae buds and larvae, which allow dispersal and settlement in new environments.

My research hypotheses are following:

1. Environmental stress (ES) experienced by hydroid colonies may affect initial size and maturity of medusae ("parental effect")
2. ES affects modes and rates of reproduction in medusae. The production of motile larvae should be promoted in adverse conditions (e.g. high population density, deterioration of conditions with progress of the season), as it allows dispersal and searching for new environment. Production of medusae buds should be promoted in favorable conditions (e.g. low population density, low salinity, medium salinity), because it allows rapid propagation and colonization new environment.
3. ES may affect maturity and reproductive rates in consecutive generations of medusae, due to side effects of vegetative reproduction.
4. ES promotes senescence in medusae
5. ES affects growth of hydroid colonies
6. ES affects production rates of medusae by hydroid colonies. There may be a trade-off between production of more hydranths (polyps) attached to colony or production of medusae, which can disperse.
7. Hydroids and medusae differ in resistance to ES, which may be an outcome of different expression of heat shock proteins

I tested these hypotheses in five separate experiments. In the first experiment (Experiment I) I investigated the effect of a combination of salinity regimes: 1) experienced by hydroid colony and 2) experienced by medusa buds, on the initial size, onset of reproduction (maturity), reproductive rates, and survival of medusae. The formation of the three generations of medusae through their asexual reproduction allowed me to investigate changes in reproductive rates with

generations. The experiment is described in Chapter 2 (2.4.1 Experimental setup) and Chapter 3 (Results for Experiment I).

The second experiment (Experiment II) was similar as the previous one. In this experiment I investigated the effect of a combination of temperature regimes on initial size, maturity, reproductive rates and survival of medusae. Experiment II was divided into two Sub-experiments separated by 3-year period. In Sub-Experiment 1, I investigated effects of medium and low temperatures on maturity, reproduction and survival of three generations of medusae. In Sub-Experiment 2, I investigated effects of medium and high temperatures on maturity, reproduction and survival of the first generation of medusae. Both sub-experiments are described in Chapter 2.4.2 (experimental setup) and Chapter 4 (Results for Experiment II). The results of the experiment were submitted to Estuaries and Coasts ("Salinity effects on survival and reproduction of hydrozoan *Eleutheria dichotoma*", Authors: Aleksandra Dańko, Maciej J. Dańko, Ralf Schaible).

In the third experiment (Experiment III) I investigated the effects of the three levels of population density on reproduction and survival of medusae. I conducted a long-term experiment with a relatively large sample sizes that allowed for the examination of age-specific mortality and reproductive rates in a gradient of population density. The experiment is described in Chapter 2.4.3 (experimental setup) and Chapter 5 (Results for Experiment III). The results were published in Marine Biology (Dańko et al. 2018a).

Finally, in the fourth Experiment (Experiment IV) I investigated the effects of the combination of different temperatures and salinities on colony growth, production of medusa buds and size of hydranths. The experiment was described in Chapter 2.4.4 (experimental setup) and Chapter 6 (Results for Experiment IV).

2 Materials and methods

2.1 Culturing conditions

The stem culture of *Eleutheria dichotoma* of the strain Ω was maintained at a temperature 23°C and in a 16 D : 8 L regime provided by incubators (RUMED 3001 and BINDER). As the source of light, Osram Lumilux Cool Daylight lamps (L18W/865) were used. The light intensity was around 7-20 μmol/m^2/s (PFD = Photosynthetically Active Photon Flux Density). These conditions provided a low-intensity light level that resembles the illumination conditions experienced by *E. dichotoma* in its natural environment. The laboratory culture was maintained in salinity 35, which reflects the average salinity of marine waters. The culturing medium was prepared from artificial ocean salt (Aquarium Systems Crystal Reefs) and MilliQ water. To reduce the number of foraminifers and to avoid the production of biofilm in culturing dishes, the medium was filtered through paper filters (0.5 μm pore diameter, Roth). The colonies of polyps were reared in 70 ml of medium in glass Boveri dishes, covered with a transparent lid in order to prevent evaporation. The dishes were changed once per month in order to prevent growth of the biofilm. The animals were fed *ad libitum* two times per week newly hatched *nauplii* of brine shrimp (*Artemia salina)*. Medium was changed one hour after feeding.

2.2 Origin of the stem culture

The original stem culture was collected by Schierwater from Banyuls-sur-Mer on the Mediterranean coast of France (Schierwater and Hauenschild 1990; Ender 1997) and was cultured for many generations in the Laboratory of ITZ Ecology and Evolution, Tierärztliche Hochschule Hannover, Germany. Since 2010, the sample has been donated to the Laboratory of Evolutionary Biodemography at the Max Planck Institute for Demographic Research in Rostock. The culturing conditions were modified following Hauenschild (1956), Schierwater (1989) and Ringelhan (2015).

2.3 Preparation of the samples and performing the measurements

2.3.1 Preparation of hydroid colonies for different experiments

For each experiment always new hydroid colony was raised. Planula larvae, which initiated each hydroid colony were collected from a stem culture and reared in Boveri dish at standard conditions (temperature 23°C, salinity 35) (20-30 larvae in a dish). After settling on the glass bottom, larvae metamorphosed into primary polyps within one week. Primary polyps were fed nauplii of *Artemia salina* that had been crushed. When the first branch of the stolon appeared (at the age of one to two weeks), polyps were fed living nauplii of *Artemia salina ad libitum*. Depending on experimental setup, the colonies were used at the age of one to four months. Increasing the rate of feeding resulted in faster growth of colonies and production of higher number of polyps, which produced more medusae. The medusae that were produced during acclimation period were removed before beginning of each experiment. From the beginning of each experiment hydroid colonies were checked daily, which allowed assembling one-day old medusae suitable for experimental treatments. Depending on experiment hydroid colonies were fed two or three times per week.

2.3.2 Checking vessels with hydroid colonies during experiments (Experiment IV)

The colonies were checked twice per week. The daily check constituted of counting and removing medusa buds, counting polyps, counting number of stolonal branches and photographing the colonies (once per week). The pictures were used to estimate growth of the colonies and size of tentacle knobs on hydranths (polyps) (**Fig. 1.2**). After checking, hydroid colonies were fed *Artemia sp. ad libitum*. Medium was changed one hour after feeding.

2.3.3 Checking vessels with medusae during experiments (Experiments I, II, III)

Experimental medusae were placed individually in single wells of six-well polycarbonate plates. Depending on experimental setup medusae were checked under dissecting microscope

(two or three times per week) for embryos present in a brood pouch (sexual maturity), newly released buds and/or planula larvae. The buds and larvae were counted and removed with a glass pipette. The first buds that fully separated from the primary medusae (medusae that had detached directly from hydroid colonies) initiated the second generation of medusae. These medusae were photographed and placed on another six-well plate. The analogous procedure was used to initiate the third generation of medusae. In order to prevent evaporation leading to increase of salinity levels, the plates were covered with a transparent lid. Despite this procedure, there were accidental cases of medium evaporation from individual wells. When evaporation was low, leading to the low decrease of medium volume, medium was fully exchanged. When medium volume highly decreased due to high evaporation, medusae were removed from the experiment (lost cases).

2.3.4 Measuring size of medusae (Experiments I, II, III)

The newly produced medusae of the first, second, and third generation, as well as sexually mature medusae were photographed. The diameter of the umbrella (the distance between the two opposite ocelli, **Fig. 2.1**) and the diameter of the buds developing on the medusae were measured by software Image J (NIH), and have been used to calculate the area of the medusae.

In order to estimate the sizes of the one-day-old vegetative medusae, I analysed the area of the body of each animal, calculated according to Schierwater (1989) as:

$$\text{Area}_{veg} = 2.622 \times \text{Umbrella diameter}^{2.26} + \Sigma\,(0.265 \times \text{Bud diameter}^{2.239})\ [\mu m^2]$$

The formula was updated for sexually mature medusae by including the area of six tentacle knobs:

$$\text{Area}_{sex} = 2.622 \times \text{umbrella diameter}^{2.26} + 6 \times (714.9 \times \text{tentacle knob diameter}^{0.955})\ [\mu m^2]$$

where umbrella diameter is a distance between two opposite ocelli placed on aboral side of umbrella and bud diameter is diameter of the buds developing on external rim of umbrella (**Fig. 2.1** and Fig. **1.4**). This approach is suitable for estimating the size of very small organisms (Schierwater 1989).

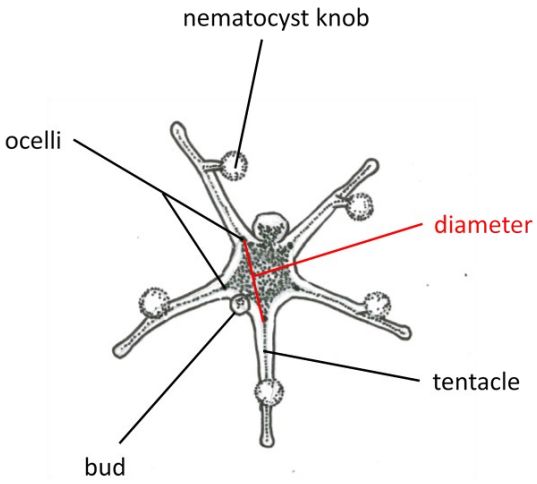

Figure 2.1 One-day-old medusa of Eleutheria dichotoma from the aboral side view. About 25-fold magnification.

2.3.5 Measuring diameter of tentacle knobs in hydranths (Experiment IV)

All experimental hydroid colonies were photographed roughly once per week. The pictures were used to measure diameter of tentacle knobs in hydranths (see **Fig. 1.3**). Diameter of tentacle knobs is not only a good indicator of the total size of medusae (Schierwater 1989), but it correlated with size of hydranths during my personal observations. Diameter of the three randomly chosen tentacle knobs was measured, from different hydranths in each experimental colony. The average diameters of tentacle knobs for each colony during each week of measurements were compared (the results are presented in Chapter 6, **Table 6.1**).

2.3.6 Measuring size of the larvae (Experiment III)

Every second week, starting on the day when most of the medusae had reached sexual maturity (around day 50 in each experimental cohort), the random sample of larvae (from 10 to 30) from each experimental regime were photographed under high magnification. The length (longer dimension) and the width (shorter dimension) of the larvae were later measured (the results are presented in Chapter 5, **Fig. 5.5**).

2.3.7 Estimating levels of heat shock proteins (HSPs) (Experiment IV)

The medusae used for HSP analysis were sampled during the main experiment and then reared in Boveri dishes in the same conditions as their parental hydroid colonies. Before preparation of the samples for HSP, medusae were starved for 48 hours. In order to minimize uncontrolled salt addition to samples, medusae were rinsed in MilliQ water. Subsequently, they were transferred to 1.5 mL micro-centrifuge tubes, weighted and homogenized with the same amount of RIPA buffer (0.15 M NaCl, 1% deoxycholate Na salt, 1% Triton X-100, 0.1 % SDS, 0.01 M Tris-HCl, pH 7.2) containing complete protease inhibitors (Roche, Basel, Switzerland, Cat. No.11836153001). All the procedures were carried out on ice in order to prevent proteolysis. The homogenates were centrifuged for 30 seconds in 30 000 rcf. Subsequently, 5µl of each supernatant from each sample was frozen on micro-centrifuge plate and were used to measure protein concentration with a DC Protein Assay (Bio-Rad, Munich, Germany, Cat.No. #500-0001), which ensured equal protein loading. The remaining supernatants were transferred to fresh micro-centrifuge tubes, and an equal volume of SDS-PAGE loading buffer (Sigma-Aldrich, Munich, Germany, Cat.No. S 3401) was added. After boiling for 5 minutes, samples were cooled down and stored in -20°C. The procedure of the protein extraction was adapted from Pijanowska and Kloc (2004).

The similar procedure was carried out for hydroid colonies. Two-months old hydroid colonies were removed from the dishes, rinsed in MilliQ water, and transferred to 1.5 mL micro-centrifuge tubes. They were mechanically homogenized in RIPA buffer containing complete protease inhibitors (Roche, Basel, Switzerland, Cat. No.11836153001) and their protein extraction followed the procedure used for medusae. The samples containing extracted proteins from hydroid colonies were stored in -20°C.

The protein concentrations measured in each sample (using DC Protein Assay) were used for dilutions of the defrosted samples with a loading buffer in order to obtain equivalent amount of protein in each sample. The samples containing equivalent amounts of protein were separated by electrophoresis on 25% SDS-polyacrylamide gels, and HSPs were immune-detected by western blotting with a primary antibody Anti-Hsp70 (Enzo, Cat.No.ADI-SPA-812-D), made by Enzo (Lorrah, Germany) and anti-GAPDH (Cat. No. G9545-100UL), made by Sigma Aldrich (Munich, Germany). The blots were then incubated with alkaline phosphatase-conjugated secondary antibody (Anti-Rabbit IgG, polyclonal antibody, Enzo ENZ-ABS257-5000), and positive immunoreaction was identified by a color reaction with SIGMAFAST^{TM} BCIP/NBT substrate (Sigma-Aldrich, Cat.No. B5655). The processed blots were examined with ImageJ (NIH, USA).

The relative amounts of the individual HSPs in each sample were calculated as a percentage of the intensity of the corresponding HSP bands in a thermally stressed human HeLa cell positive control sample (Enzo, Cat.No. ADI-LYC-HL101-F) run alongside the test samples

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2.4 Experimental setups

2.4.1 Effects of salinity on reproduction and survival of medusae in hydrozoan *Eleutheria dichotoma* (Experiment I)

2.4.1.1 Test study

A gradient of regimes (salinity 15 – 50) was used in a preliminary experiment, with a "control" (medium) salinity of 35. The preliminary experiment showed that at salinities on either side of the control, i.e. 25 and 45, the medusae still had buds and survived the whole observation period (45 d). Medusae maintained outside this range (in salinities of 15, 20, and 50) soon died without producing any buds or larvae. Based on these results, three levels of salinity were applied in the main experiment: 25 (low salinity), 35 (medium salinity), and 45 (high salinity). The lowest salinity reflects conditions that may occur after heavy rain, while the highest reflects conditions in isolated, shallow lagoons (Metaxas and Scheibling 1993) exposed to evaporation (Damgaard and Davenport 1994).

2.4.1.2 Preparation of the experimental hydroid colony

Using three different larvae randomly collected from a stem culture, I raised three hydroid colonies and cultured them for four months (July - October 2014) in standard conditions (temperature 23°C, salinity 35). After this period, each colony was cut into three fragments containing the same number of hydranths. Each fragment of the colony was randomly assigned to one of three salinity regimes (25, 35, 45), resulting in three replicates for each salinity. In order to increase the number of hydranths in colonies, each hydroid colony was fed three times per week using naupli of *Artemia* sp. *ad libitum*. In the week before the experiment, the feeding regime was changed to twice a week, which was further continued through the experiment for all colonies and medusae. The three fragments of one colony maintained in different salinities were randomly chosen as a source of experimental medusae produced in salinities 25, 35 and

45. The medusae used to initiate the experimental treatments were assembled during the same period of the year (October - December 2014). As hydroid colonies may differ in reproductive activity over the year (Coma et al. 2000; Bavestrello et al. 2006), we avoided the possibility of variation in rates of medusa buds production between experimental colonies. The experiment on three generations of medusae has been completed in July 2015.

2.4.1.3 Scheme of the experiment

Before the beginning of the experiment, all of the medusae released by hydranths during the acclimation period were removed. From that point forward, the three fragments of the experimental hydroid colony were checked every day for newly released medusa buds (primary medusae) and were fed two times per week. One-day-old medusae were photographed and randomly assigned to one of the seven experimental treatments (**Fig. 2.2**). In the high salinity treatments, I collected fewer medusae, and these medusae reproduced at a lower rate. I was, therefore, unable to collect medusae of further generations from these treatments.

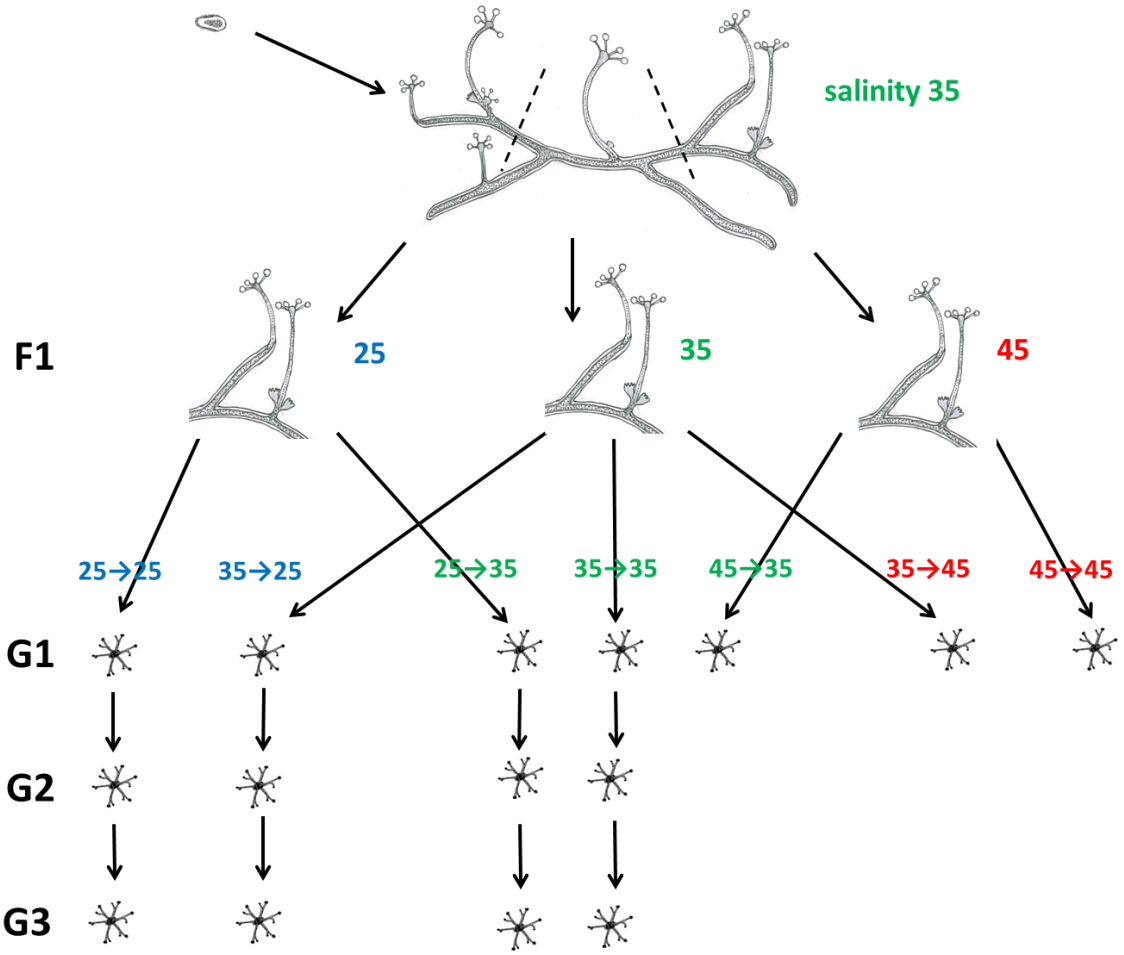

Figure 2.2 Scheme of the experiment; Experimental protocol to test the effects of salinity on the reproduction and survival of hydrozoan Eleutheria dichotoma. The hydroid colony was divided into three parts with equal numbers of hydranths (polyps). Each part was acclimated to a different salinity level (low 25, medium 35, or high 45). The medusae assembled from the hydroid colonies that were reared in marginal salinities (low or high) were either maintained in the same salinities (regimes P25M25, number of individuals: G1=36, G2=36, G3=36 and P45M45, G1=41), or transferred to the medium salinity (regimes P25M35, G1=36, G2=32, G3=28 and P45M35, G1=12, G2=11). The medusae released by the hydroid colonies in the medium salinity were either reared in the same conditions (regime P35M35, G1=42, G2=40, G3=36), or transferred to one of the marginal salinities (regimes P35M25, G1=36, G2=33, G3=33 and P35M45, G1=21). There was no transfer of the medusae from low to high salinity conditions.

The medusae were reared individually in 9.5 mL of salt water in single wells of six-well polycarbonate culture plates with transparent covers minimizing evaporation. They were fed three to four naupli of *Artemia* sp. twice per week. Change of the medium was performed once per week, after feeding, by moving medusae to the plates with a newly prepared salt water. Three times per week, the medusae were checked under a dissecting microscope for newly released buds and/or planula larvae. The buds and larvae were counted and removed. The first

buds that fully separated from the primary medusae initiated the second generation. The same procedure was used to initiate the third generation. The newly produced medusae of the first, second, and third generation were photographed. The diameter of the umbrella (the distance between the two opposite ocelli) and the diameter of the buds developing on the medusae were measured using software ImageJ (NIH). These measurements were used to calculate the area of the medusae (see description of the method in chapter 2.3.2).

The medium in the wells was changed once per week, but the level of medium in the plates with medusae were checked three times per week. In some cases, moderate evaporation was observed in individual wells. When a well was identified as having low volume from evaporation, the medium was fully exchanged. Any death that occurred in these conditions (12% of lost replicates), were treated as lost (right censoring, see also statistical methods).

In order to document the changes in morphology of the medusae over their lifespans, six medusae of the first generation from each experimental cohort were photographed once per week (the representative pictures are collected in supplementary materials, **Fig. A3**).

2.4.1.4 Statistical methods

All of the statistical analyses were performed in R language (R-Core-Team 2018). Age at asexual and sexual maturation, as well as age at death, are time-to-event data. I analysed these data using methods typical in survival analysis. The data were visualized using a Kaplan-Meier estimator of survival (or the inverse of it for maturity data) (Kaplan and Meier 1958). Medusae were lost from the study for two main reasons: (1) laboratory accidents, like water evaporation; and (2) the medusa was absorbed by a larger medusa bud (supplementary materials **Fig. A2**). The lost cases were considered to be right censored (Klein and Moeschberger 2003; Kleinbaum and Klein 2012). I assumed that all losses occurred in the middle of an interval between two consecutive observations. The medusae that released the first buds were treated as asexually

mature, whereas the medusae with fully developed embryos and/or the medusae that had released larvae for the first time were treated as sexually mature.

To analyze age at maturity, I used a semi-parametric test: the Cox proportional hazard model (survival R-package; (Therneau and Grambsch 2013)). The proportional hazard assumption was tested visually by inspecting the log cumulative hazard (log(-log[Survivorship]) plots for each combination of salinity treatments (polyps and medusae) and generations. The most parsimonious models were selected using a progressive hierarchical likelihood ratio test. In this test, I started with a model with no interactions between the categorical predictors, but with all the main effects present. I then sequentially added the interactions and tested their significance. For both kinds of maturity data (asexual and sexual), I found that the models without interactions were the most parsimonious.

As the proportional hazard assumption was clearly violated for the survival data, I avoided using Cox regression, and instead applied two non-parametric tests for the equality of survival distributions: the log-rank test (Mantel 1967; Harrington et al. 1982); survival R-package (Therneau and Grambsch 2013) and the Gehan-Breslow-Wilcoxon test (Gehan 1965; Breslow 1970); coin R-package (Hothorn et al. 2008). The log-rank test is most powerful when the hazard ratios are proportional, but it is mainly sensitive to late-occurring events. The Gehan-Breslow-Wilcoxon test is more powerful than the log-rank test when the proportional hazard assumption is not fulfilled, but it gives more weight to early events (Martinez and Naranjo 2010). I used the Gehan-Breslow-Wilcoxon test as an additional test because it can partially complement the log-rank test.

I observed that the salinity conditions experienced by the hydroid colonies had no or minimal effects on survival of medusae in consecutive generations. There was still no effect when the generations were pooled (see **Tab. A1** in supplementary materials). I therefore decided to combine the time-to-event data from experiments in which the medusae experienced

the same conditions independent of the conditions experienced by hydroid colonies. The effects of the generation of the medusae for the time-to-event data were analyzed separately for each salinity regime. The effects of salinity were analyzed separately for each generation of medusae, and for combined generations. The p-values were corrected for multiple-comparison bias using Holm's method.

Asexual and sexual daily reproduction rates were analyzed using general linear models (GLM). To avoid biased statistical inferences caused by overdispersion, I used the negative binomial regression (MASS R-package; Venables and Ripley 2002)). In both sets of models, the sum of the released medusae and/or larvae was set as the dependent variable; the natural logarithm of individual exposures (individual days lived) was set as the offset; and the natural logarithm was set as the link function. I decided to remove the 45 treatments (for both the hydroid colonies and the medusae) from the reproduction rate analyses because these treatments contained groups for which there were no counts and no data for the second and third generations. A comparison of the P35M35 group with the P45M35 group (high polyp salinity of 45, medium medusa salinity of 35) was done separately using a negative binomial model. The most parsimonious model was selected from the partially nested models constructed from different combinations of main effects and their interactions by minimizing the Akaike information criterion (AIC) (Burnham and Anderson 2003). Insignificant interactions were dropped from the model.

The age-specific reproduction rates were smoothed by fitting one-dimensional Poisson penalized splines (MortalitySmooth R-package; Camarda 2012) that accounted for over-dispersion. The log of exposures was set as an offset, and the smooth parameter lambda was selected via the minimization of the BIC (Bayesian information criterion). The standard errors of the reproduction rates were calculated from the standard errors of the smoothed log rates.

The 95% confidence intervals were calculated from the standard errors following the normality assumption.

2.4.2 Effects of temperature on reproduction and survival of medusae in two clones of *Eleutheria dichotoma* (Experiment II)

2.4.2.1 Experimental design

In a distance of the three years I conducted two separate sub-experiments investigating influence of the temperature on reproduction and survival of medusae *Eleutheria dichotoma*. I used three different temperatures to test responses over the range of local conditions in north Mediterranean: 12°C (low temperature, representing cold winter in north Mediterranean); 28°C (high temperature, representing hot summer in north Mediterranean) (Purcell et al. 2012), and 23°C (medium temperature, control conditions).

2.4.2.2 Sub-experiment 1: Effects of low (12°C) and medium temperature (23°C) on reproduction and survival of three generations of medusae

Hydroid colony, which was source of experimental medusae was raised from one planula larva and reared for three months at standard conditions (temperature of 23°C, salinity 35). Subsequently, the colony was divided into two fragments containing the same number of hydranths (polyps). One fragment of the colony was maintained further in 23°C, and another fragment was transferred to 12°C (**Fig. 2.3**).

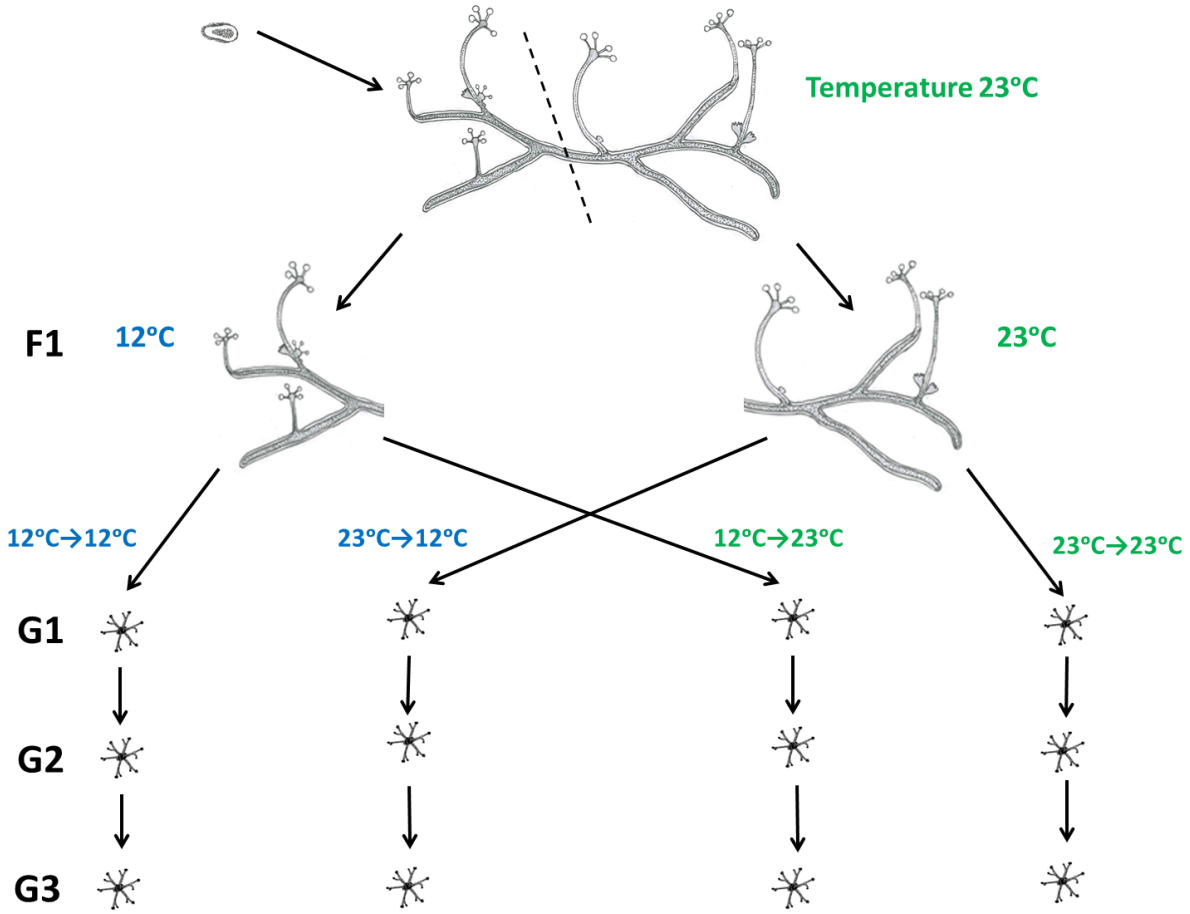

Figure 2.3 Scheme of the experiment. Experimental protocol to test the effects of temperatures 12°C and 23°C on reproduction and survival of medusae Eleutheria dichotoma. The hydroid colony was divided into two fragments with equal number of hydranths (polyps). Each part was acclimated to a different temperature (low 12°C and medium 23°C). The medusae assembled from hydroid colonies that were reared in low or medium temperature were either maintained in the same temperature (regimes P23M23 and P12M12) or transferred to different temperature (regimes P12M23 and P23M12). Three generations of medusae were tested in this study.

After three weeks of acclimation, all medusae produced during this period were removed. Since then, the two fragments of hydroid colony were checked every day in order to collect one-day old medusae starting experimental first generation (primary medusae). One-day old medusae were photographed and assigned to experimental treatments by alteration. Medusae were maintained individually in 9.5 mL of salt water on 6 - well polycarbonate plates and fed 3 - 6 naupli of *Artemia* sp. three times per week. The number of buds and larvae were counted for each medusae before feeding. Buds and/or planula larvae were removed. Medusae with embryos present in a brood pouch were photographed and the pictures were used for

measurement of their size at sexual maturity. Medium was changed once per week after feeding, by transferring medusae to the new plates with a fresh medium. Second generation of medusae constituted of the first bud fully separated from primary medusae. The same procedure was used to initiate the third generation. Experiment was carried out from February 2014 - March 2015.

2.4.2.3 Sub-experiment 2: Effects of high (28°C) and medium temperature (23°C) on reproduction and survival of the first generation of medusae (primary medusae)

Experiment was carried out three years later, from October 2015 to September 2016. Using three different larvae randomly collected from a stem culture, I raised three different hydroid colonies. The three hydroid colonies were maintained at temperature of 23°C and salinity 35 for two months and then divided into two fragments containing the same number of hydranths (polyps) (**Fig. 2.4**). One fragment of each colony was maintained further in temperature of 23°C and another fragment was transferred to 28°C.

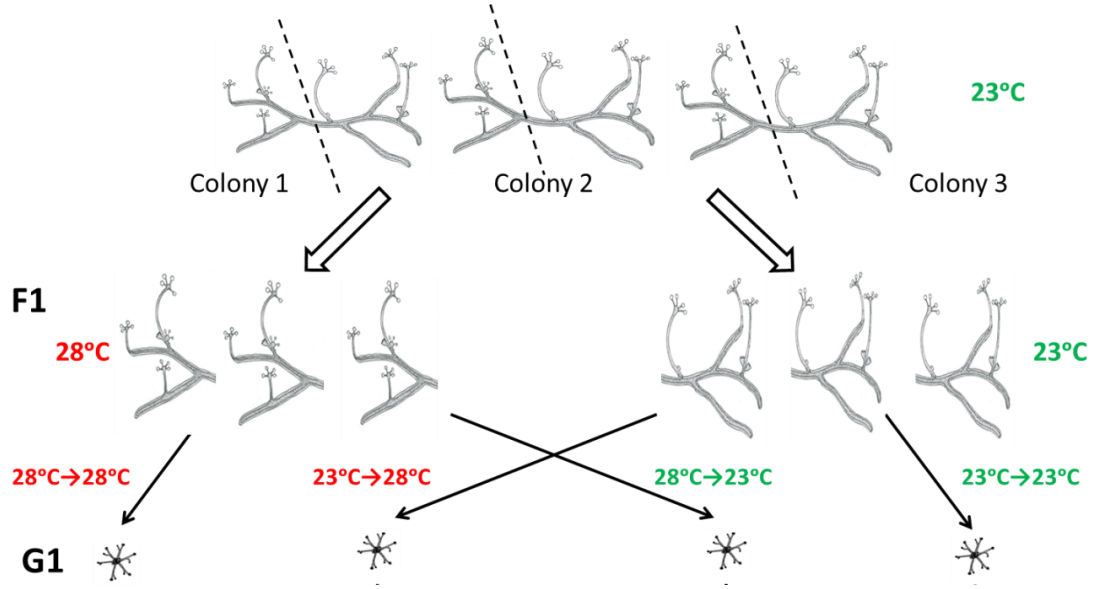

Figure 2.4 Scheme of the experiment. Experimental protocol to test the effects of temperature 28°C and 23°C on reproduction and survival of hydrozoan Eleutheria dichotoma. The three hydroid colonies raised from different planula larvae were divided into two fragments with equal number of hydranths (polyps). Each part was acclimated to a different temperature (high 28°C and medium 23°C). The medusae assembled from hydroid colonies that were reared in high or medium temperature were either maintained in the same temperature (regimes P23M23 and P28M28) or transferred to different temperature (regimes P28M23 and P23M28). Only the first generation of medusae (primary medusae) was investigated.

After three weeks of acclimation all medusae produced by hydroid colonies during this period were removed. Since then, the colonies were checked every day and medusae were assembled to start experimental first generation (primary medusae). Collecting medusae from three different hydroid colonies allowed us to obtain many medusae in a short time. One-day old medusae were photographed and assigned to experimental treatments by alteration. Medusae were maintained individually in 9.5 mL of salt water on 6-well polycarbonate plates and fed 3 – 6 naupli of *Artemia* sp. two times per week. Before feeding the wells were checked for new buds and/or larvae, which were subsequently removed. Medusae with embryos present in a brood pouch were photographed and the pictures were used for measurement of their size at sexual maturity. Medium was changed once per week after feeding, by transferring medusae to the new plates with a fresh medium.

2.4.2.4 Statistical methods

All of the statistical analyses were performed in R language (R-Core-Team 2018). Age at asexual and sexual maturation as well as age at death are time-to-event data, and were analyzed using methods that are typical in survival analysis. The data were visualized using a Kaplan-Meier estimator of survival (or the inverse of it for maturity data). Medusae were lost from the study for two main reasons: (i) laboratory accidents, like water evaporation, etc.; and (ii) events of the absorption of the medusa by a larger medusa bud. Such losses were considered as right censored. I assumed that all events occurred in the middle of an interval between two consecutive observations. The medusae that released the first buds were treated as asexually matured, whereas the medusae with fully developed embryos and/or the medusae that had released larvae for the first time were treated as sexually matured.

As the proportional hazard assumption was clearly violated for the maturity and survival data, I avoided using Cox regression, and instead applied two non-parametric tests

for the equality of survival distributions: the log-rank test (survival R-package: Therneau and Grambsch 2013 and the Gehan-Breslow-Wilcoxon test (Gehan 1965; Breslow 1970); coin R-package: Hothorn et al. 2008). For more details see 2.4.1.4 in the Experiment II

Asexual and sexual daily reproduction rates were analyzed in the same way as in Experiment II, using negative binomial regression. For more details see 2.4.1.4 in the Experiment II.

The diameter of the umbrella (the distance between the two opposite ocelli) at birth and first sexual reproduction were made with software ImageJ (NIH). These measurements were used to calculate the area of the medusae (see description of the method in chapter 2.3.2).

The data were analyzed by linear regression, assuming $\log_{10}$ of area as a dependent variable. The log transformation was necessary to obtain normally distributed residuals.

2.4.3 Effects of population density on reproduction and survival of medusae in hydrozoan *Eleutheria dichotoma* (Experiment III)

2.4.3.1 Experimental design

To create a large sample of primary medusae of the same age within a short period of time, I assembled medusae from four colonies of polyps. The colonies were checked every day for newly released primary medusae. All of the medusae produced by the four colonies of polyps were assembled into one collective Boveri dish. Next, single medusae were randomly distributed to wells on polycarbonate plates representing different experimental regimes (**Fig. 2.5**).

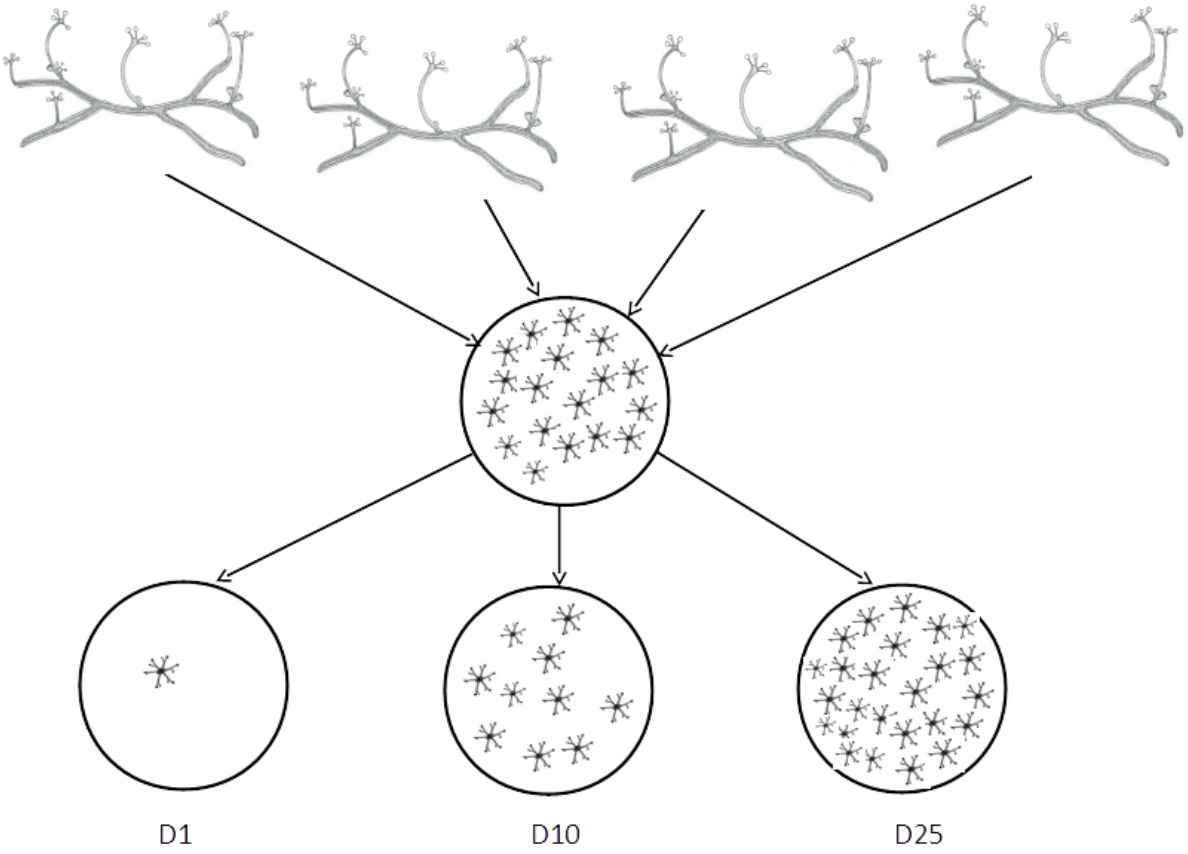

Figure 2.5 Scheme of experiment investigating effects of population density on reproduction and survival of medusae. Medusae produced by four polyp' colonies were collected in one assembling dish. Further, they were randomly attributed to experimental regimes (one individual in a well, 10 individuals in a well and 25 individuals in a well).

The experiment consisted of three combinations of treatments:

D.1: 1 medusa in a well (6*15 plates = 90 ind.)

D.10: 10 medusae in a well (11 wells = 110 ind.)

D.25: 25 medusae in a well (5 wells = 125 ind.)

The medusae were photographed in experimental wells on their day of birth, and again on the day they reached sexual maturity (when embryos were noticed inside the umbrella). All of the experimental wells with medusae were checked under a binocular three times per week. The total number of medusae in each well was recorded, and the newly released buds were removed.

It was sometimes difficult to distinguish between the newly released buds and the individual medusa. I based the distinctions I made on my earlier observations. Buds are usually smaller and more orange than the medusa mother, and they generally do not have embryos in the umbrella (see the supplementary materials, **Fig. A7**). The medusae were checked for the presence of embryos inside the umbrella. Free-swimming planula larvae, which were released after their initial development as embryos inside the umbrella, were counted and moved to the assembling dish for each separate regime. Each medusa from each experimental regime was directly fed two-day-old *nauplii* of *Artemia salina ad libitum* three times per week. The medusae that were fully dissolved after a period of gradual deterioration were considered dead. The dead medusae were not replaced. The experiment was finished when all of the medusae in a well died (dissolved).

2.4.3.2 Statistical methods

All of the analyses were performed in the R language and environment (R Core Team 2015). The survivorship was analyzed using the Kaplan-Meier estimator (Klein and Moeschberger 2003). The medusae that were lost or absorbed by a bigger bud were treated as censored. The equality of the survival distributions was tested using a log-rank test (Mantel 1967; Cox 1972) (survival R-package). The trend in survival distributions was analyzed using a Gehan-Mantel Trend Test (Leissen et al. 2009).

The empirical mortality data were fitted with γ-Gompertz and γ-Gompertz-Makeham models (Gompertz 1825; Makeham 1867; Vaupel et al. 1979; Missov et al. 2016; Dańko et al. 2017b) by means of a maximum likelihood approach. The hazard rate for γ-Gompertz-Makeham is defined according to the formula

(i) $$\mu = \frac{ae^{bx}}{1+\frac{a\gamma}{b}\left(1-e^{bx}\right)} + c$$

where a is initial age-dependent mortality, b is the rate of aging, γ is heterogeneity parameter, and c is age-independent mortality. For c=0, the model is simplified to a γ-Gompertz model, and a becomes the intercept of the mortality curve. For each of the populations, I checked the significance of the Makeham term using a likelihood ratio test (LRT). In this case, a pair of models was compared, with one model being nested within the other. Because the null hypothesis c=0 lies at the boundary of the parameter space, I corrected the p-values by dividing them by two (Ota et al. 2000; Pietrzak et al. 2015). As it was found that Makeham term c was insignificant in all cases (D1: ratio=0, p=0.5; D10: ratio=0.5885, p=0.2215; D25: ratio=0.3571, p=0.2751), γ-Gompertz for the rest of the analyses was used.

Similarly, a LRT was used to compare the γ-Gompertz parameters in the three studied populations. A LRT is typically used to compare a pair of models in which one model is nested within the other. Here, an extended model for three populations was constructed (Pletcher 1999). A null hypothesis assumes that one of the corresponding parameters is fixed for all populations, while the alternative hypothesis assumes that each parameter is fitted independently.

The lifetime mean reproductive rates were analyzed using the GLM with Poisson errors. In the model, the total number of buds as a count-dependent variable was included, and the log of exposures was calculated as a sum of the lifespans of individuals (as an offset). The normality of the residuals was checked using quantile-quantile plots.

The age-specific reproductive rates for both sexual and asexual reproduction were smoothed by fitting one-dimensional Poisson penalized splines (P-splines). The procedure was performed using the *MortalitySmooth* R-package (Camarda 2012). The optimal values of the smoothing parameter were selected using the BIC model selection, and the fitting allowed for the potential over-dispersion. Standard errors for the reproduction rates were calculated using the delta method from the obtained standard errors of the smoothed log-reproduction rates. The

piecewise confidence intervals for the fitted reproductive rates were calculated from the abovementioned standard errors with a normality assumption.

The diameter of the umbrella, the diameter of the buds, and the diameter of the tentacle knobs were used to calculate the two-dimensional surface area of the asexual and sexual medusae, which is a better indicator of size for very small organisms (Schierwater 1989) (see description of the method in chapter 2.3.2). The data were analyzed by linear regression, assuming $\log_{10}$ of area as a dependent variable. The log transformation was necessary to obtain normally distributed residuals.

In the analysis of the sizes of the larvae, the length and the width were treated as multivariate dependent variables, and the density was treated as a continuous covariate. The analysis was performed with MANCOVA (*car* R-package, Pillai method), assuming a type III sum of squares (significant interaction, **Fig. 5.5**)

2.4.4 Effects of temperature and salinity on reproduction of hydroid colonies in hydrozoan *Eleutheria dichotoma* (Experiment IV)

2.4.4.1 Experimental design

Experiment was carried out from May to July 2016, which overlaps with the period when animals are observed in the wild. This allows to avoid fluctuation of hydroid activity due to internal rhythms (Bavestrello et al. 2006). To investigate the effects of temperature and salinity on growth and reproductive rates of hydroid colonies, I cultured 36 colonies and later distributed them randomly in nine combinations of regimes of temperature (18°C, 23°C, 28°C) and salinity (25, 35, 45), with four replications per each regime (see supplementary materials **Fig. A8**).

I used three different temperatures to test responses over the range of local conditions in north Mediterranean: 18°C (low temperature, representing autumn or mild winter in north

Mediterranean); 28°C (high temperature, representing hot summer in north Mediterranean (Purcell et al. 2012); and 23°C (medium temperature, control conditions). I raised the hydroid colonies from different planula larvae collected from the stem culture. Both larvae and primary polyps (which metamorphosed from larvae) were reared in glass Boveri dishes at standard conditions (temperature 23°C, salinity 35). After settlement and metamorphosis of larvae, primary polyps were fed every second day crashed *Artemia* sp. All the initial steps of colonial development were recorded. Hydroid colonies at the age of two - three weeks (having stolon with one - three hydranths) were randomly attributed to nine different regimes of temperature (18, 23, and 28°C) and salinity (25, 35 and 45). Individuals for the experimental cohorts were collected gradually, as young hydroid colonies reached the expected number of hydranths within one - three weeks. The hydroid colonies were fed two times per week newly hatched *Artemia* sp. Medium in dishes was changed the day after feeding. At each feeding day, the number of hydraths, the number of stolonal branches (hydrocladia), and the number of released medusa buds were recorded. All the hydroid colonies were photographed roughly once per week. The pictures were used to measure the total length of the stolon and diameter of tentacle knobs in hydranths (see description of the method in chapter 2.3.4).

2.4.4.2 Statistical methods

All analyses were performed in R language and environment (R-Core-Team 2018).The influence of temperature (18, 23, and 28°C), salinity (25, 35, and 45), and age on length of the stolons, number of hydranths, number of branches on a stolon, as well as medusae production rate were analyzed by Generalized Additive Mixed Models (GAMM) using the *mgcv* (Woods 2017) and the *itsadug* (van Rij et al. 2015) packages. The full model assumed all possible combinations of main effects and interactions among age (smoothed), temperature, and salinity. The interactions (i) between age and temperature, (ii) age and salinity, and (iii) age, temperature

and salinity were modeled by tensor product interactions. Because each colony was nested in only one combination of treatments (four colonies in each salinity-temperature combination), a hydroid colony was treated as a nested random factor. In the models, I considered uncorrelated random intercepts and random slopes. Random effects were modeled by smooths ("re" basis; the *gam* function, the *mvgc* package). The full models were fitted with the REML (Restricted Maximum Likelihood) method and its terms were selected by using extra penalty on each term (option *select* = *TRUE* of the *gam* function of the *mvgc* package).

Dependent variables had different types, therefore different assumptions about distribution and link function were made. The length of the stolon was modeled with assumption of normal distribution and log link function. The log link helped to keep fitted values positive as well as improved the fit of the model. Number of hydranths and number of branches are count data and they were fitted with assumption of negative-binomial distribution and log link. The rate of releasing of medusae was analyzed assuming negative-binomial distribution, log link, and offset equal to the logarithm of exposures (individual-days-lived). The exposures were calculated as a sum of observed individual-days during an age interval for all hydroid colonies. I assumed that medusae were released in the middle of the measured age interval. The fit of the models were validated by the *gam.check* function of the *mvgc* package.

Multiple comparisons between pairs of age-specific patterns of a single measure (length of stolon, number of hydranths, number of branches, or medusa production rate) for combination of salinity and temperature treatments were done by model predictions with random effect of colony excluded (Woods 2017). The age-specific predicted responses for a pair of compared regimes were subtracted to find age regions significantly different from zero (van Rij et al. 2015). The inference was based on confidence intervals of the difference, which were adjusted for multiple comparisons bias. For further details see supplementary materials III and IV.

Knob diameter data has simple age patterns that did not need to use generalized additive models. I found model without any random effect as the most parsimonious, therefore selection of interactions of fixed terms were conducted on models fitted by simple linear regression. The selection was done using AIC. I found that the model without interactions was the most parsimonious one.

3 Effects of salinity on reproduction and survival of medusae in hydrozoan *Eleutheria dichotoma* – (Results for Experiment I)

3.1 Effects of salinity on age at first sexual and asexual reproduction (maturity of medusae)

Salinity influenced the age when the first medusa bud was released by a medusa (first asexual reproduction). The medusae that experienced low salinity conditions (25) generally produced the first bud (became asexually mature) at a rate that was roughly three times faster than that of the medusae reared in medium salinity conditions (35). I found that generation number had a significant effect on the rate of individuals becoming asexually mature. The rate of asexually mature medusae decreased ~ twofold between each of the consecutive generations (**Table 3.1**). In the cohorts with medusae reared in high salinity (45), individuals reached asexual maturity only in the P45M45 treatment (hydroid colonies reared in salinity 45, medusae reared in salinity 45); however, even in this case < 20% of the individuals reached maturity (**Fig. 3.1A**). There were no significant differences in the patterns of asexual maturity of the P45M35 group and the P35M35 group (log-rank: Chi square=0.64, p=0.423; Gehan-Breslow: Chi square=2.36, p=0.124).

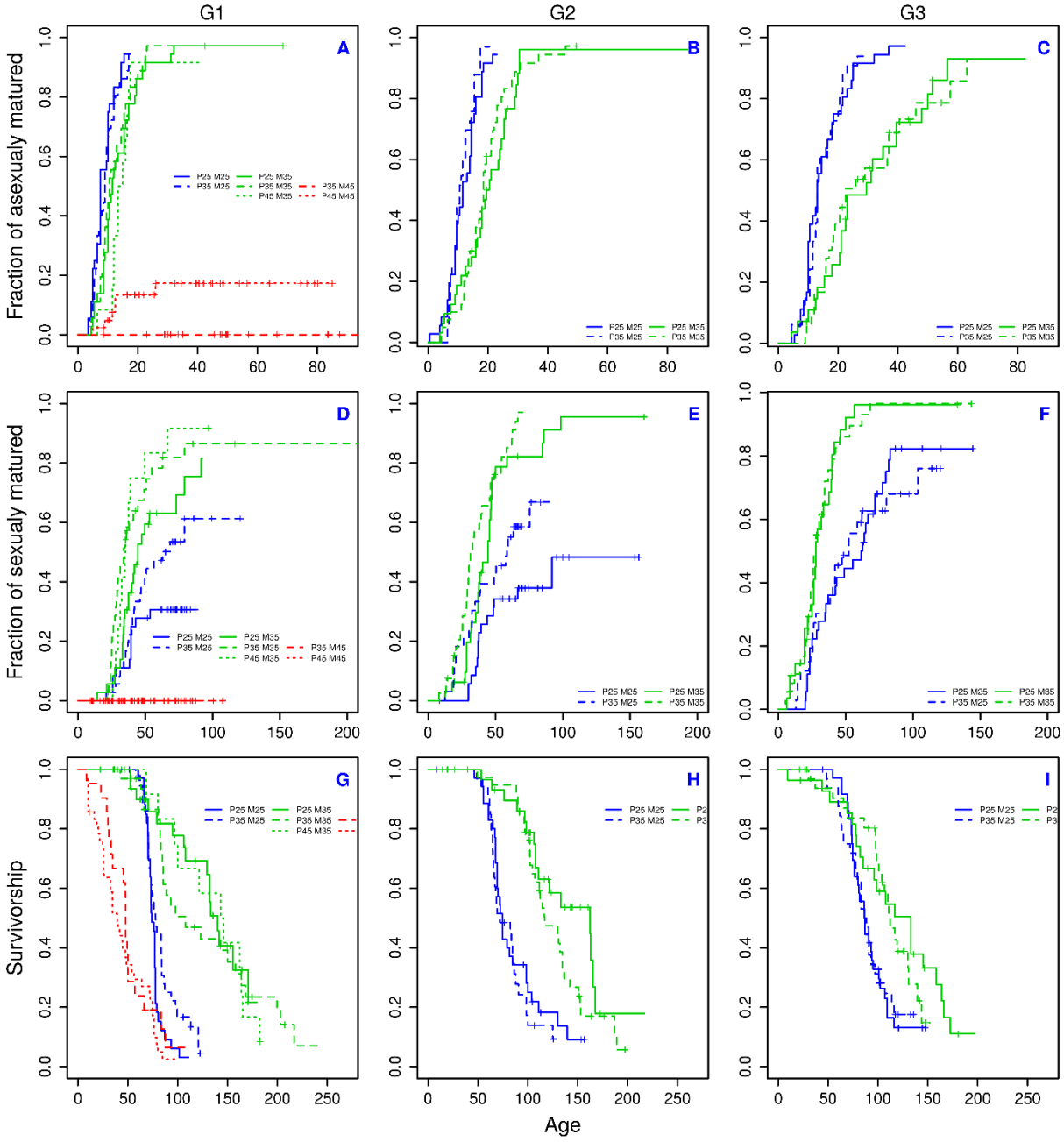

Figure 3.1 Asexual maturity, sexual maturity, and survivorship in three generations of medusae Eleutheria dichotoma maintained in different salinities. The first two rows represent the inverse of Kaplan-Meier estimators of asexual (A, B, C) and sexual (D, E, F) maturity. The third row (G, H, I) represents the Kaplan-Meier estimator survival of the medusae in the different salinities. The medusae that detached from hydroid colonies at one salinity were transferred to different salinity conditions. The line patterns represent the different salinities experienced by the hydroid colonies; the colours represent the different salinities experienced by the medusae; and the columns represent the different generations of the medusae. Age is measured in Days. Medusae from regime P35M35 were released by hydroid colony at salinity 35 and were maintained in the same salinity. Medusae from regime P35M25 were released by hydroid colonies at salinity 35 and were transferred to salinity 25. The other regimes should be read analogically.

Table 3.1 Asexual maturity of hydromedusae Eleutheria dichotoma reared in different salinity treatments; Results of the fitted Cox proportional hazard regression models. The interactions were non-significant, and were dropped from the models. The treatments with the hydroid colonies and the medusae that experienced high salinity levels were excluded from this analysis (see statistical methods). The reference was set as P35M35G1 (hydroid colony reared at salinity 35, medusae reared at salinity 35, first generation).

	coef	exp(coef)	SE	z	Pr(>|z|)
Polyp (Salinity = 25)	-0.0955	0.9089	0.1010	-0.95	0.344
Medusa (Salinity = 25)	1.0814	2.9488	0.1108	9.76	<0.001
Generation (G2)	-0.6404	0.5271	0.1221	-5.25	<0.001
Generation (G3)	-1.2484	0.2870	0.1316	-9.48	<0.001

Table 3.2 Sexual maturity of hydromedusae Eleutheria dichotoma from different salinity treatments; Results of the fitted Cox proportional hazard regression models. The interactions were non-significant, and were dropped from the models. The treatments with the hydroid colony and the medusae that experienced high salinity levels were excluded from this analysis (see statistical methods). The reference was set as P35M35G1 (hydroid colony reared at salinity 35, medusae reared at salinity 35, first generation).

	coef	exp(coef)	SE	z	Pr(>|z|)
Polyp (Salinity = 25)	-0.3142	0.7303	0.1183	-2.66	0.008
Medusa (Salinity = 25)	-1.0686	0.3435	0.1239	-8.63	<0.001
Generation (G2)	0.2799	1.3230	0.1476	1.90	0.058
Generation (G3)	0.7003	2.0144	0.1467	4.77	<0.001

Salinity influenced the age when planula larvae were observed inside medusa and/or when the first larva was released. Most of the results of the fitted model for sexual maturity were the opposite of the results for asexual maturity. The medusae that experienced low salinity conditions generally matured sexually at a rate that was roughly three times slower (0.34) than that of the medium salinity treatment. The effect of generation on maturity was significant for all treatments. The rate of sexual maturity increased 32% from G1 to G2. This rate was even higher in G3 (roughly twice as high as in G1) (**Table 3.2**). Remarkably, none of the medusae matured sexually at the highest salinity level (**Fig. 3.1B**). There was no difference in the sexual maturity patterns of the P45M35 group and the P35M35 group (log-rank: Chi square=0.01, p=0.924; Gehan-Breslow: Chi square=0.25, p=0.616).

Interestingly, I observed that the salinity conditions experienced by the hydroid colonies had a significant effect on the age when the first larva was released (**Table 3.2**), but not on the age when the first bud was released (**Table 3.1**). The medusae released by hydroid colonies in low salinity (25) had a sexual maturity rate that was 27% slower than that of the medium salinity group (**Table 3.1**).

3.2 Asexual reproduction rates (medusae budding rates)

The rates of asexual reproduction were the highest in low salinity (**Fig. 3.2** A; **Table 3.3**). However, salinity conditions experienced by hydroid colonies, as well as their interaction with the salinity experienced by medusae, had minor effects on budding rates **(Table 3.3**). Medusae that detached from hydroid colonies in low salinity and were further reared in medium salinity (P25M35) had slightly higher budding rates than medusae from treatment P35M35 (**Fig. 3.2**B). I found no differences in the budding rates of the P45M35 and P35M35 groups in the first generation ($z=-0.89$, $p=0.374$). The budding rates decreased with generation in all treatments (**Fig. 3.2**B and C; **Table 3.3**).

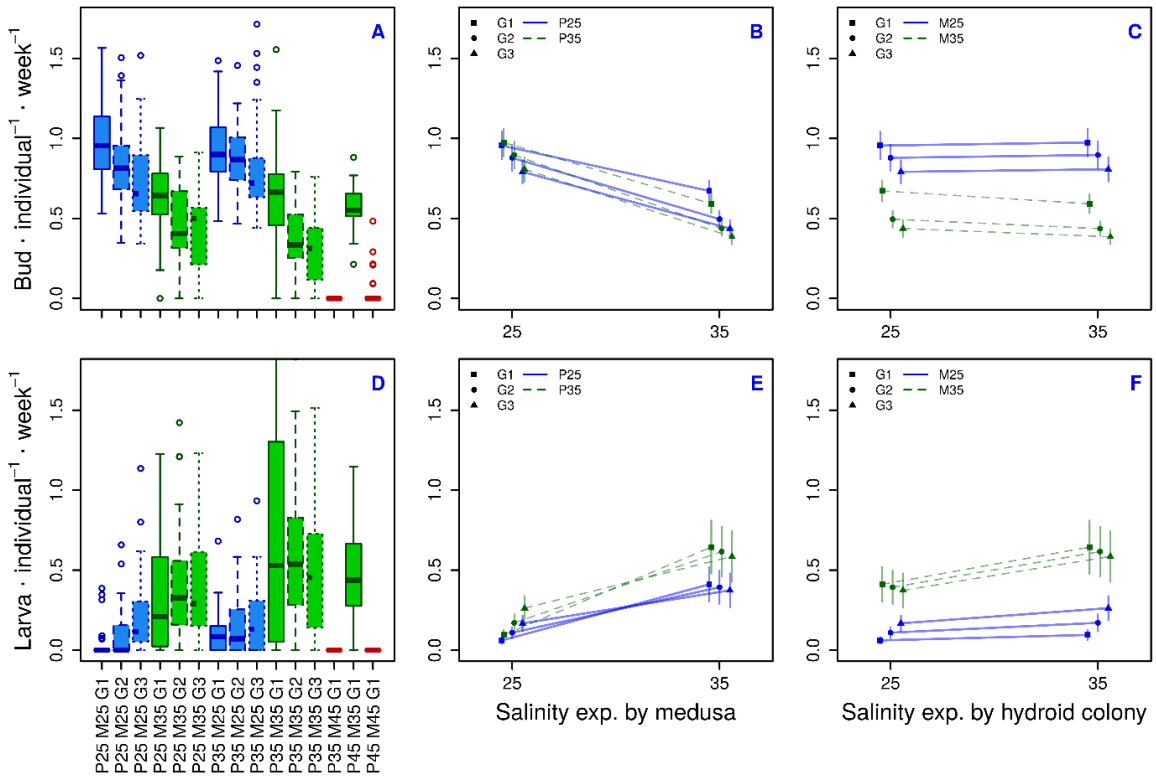

Figure 3.2 The rates of bud production (upper panels: A, B, C) and larva production (lower panels: D, E, F) in three generations of hydromedusa Eleutheria dichotoma maintained at different salinity levels. A and D: box plots of data, B and E: predictions of corresponding models shown from the perspective of the effect of salinity experienced by medusa, C and F: predictions of corresponding models shown from the perspective of the effect of salinity experienced by hydroid colony. Estimated daily rates were converted to weekly rates. Medusae from regime P35M35 were released by hydroid colony at salinity 35 and were maintained in the same salinity. Medusae from regime P35M25 were released by hydroid colony at salinity 35 and were transferred to salinity 25. The other regimes should be read analogically.

Table 3.3 Asexual reproduction rate; Results of the most parsimonious model, which was fitted assuming a negative binomial distribution. The highest applied salinities were excluded from this analysis (see statistical methods). The reference was set as P35M35G1 (hydroid colony reared at salinity 35, medusae reared at salinity 35, first generation).

	coef	**exp (coef)**	**SE**	**z**	**Pr(>|z|)**
(Intercept)	-2.4694	0.0846	0.0507	-48.66	<0.001
Polyp (Salinity = 25)	0.1261	1.1344	0.0542	2.33	0.020
Medusa (Salinity = 25)	0.4979	1.6453	0.0679	7.33	<0.001
Generation (G2)	-0.3050	0.7371	0.0625	-4.88	<0.001
Generation (G3)	-0.4303	0.6503	0.0693	-6.21	<0.001
Polyp x Medusa (Salinity = 25)	-0.1457	0.8644	0.0717	-2.03	0.042
Medusa x Generation (Salinity = 25, G2)	0.2216	1.2481	0.0845	2.62	0.009
Medusa x Generation (Salinity = 25, G3)	0.2419	1.2737	0.0898	2.69	0.007

3.3 Sexual reproduction rates (larval production rates)

I found that the salinity levels experienced by the hydroid colonies and by the medusae had significant effects on the larval production rates (**Fig. 3.2 D, E, F**; **Table 3.4**). In general, the highest rate of larval production was observed in medium salinity. The experience of low salinity at the beginning of medusa bud development (when attached to hydranth) or on the course of medusa life, was associated with lower sexual reproduction rates (**Fig. 3.2 E, F**). The effect of salinity experienced by hydroid colonies on larval production rates was smaller than for budding rates (**Table 3.4**). The medusae that experienced the highest salinity levels did not produce planula larvae. There were no differences in the larval production rates in the P45M35 and the P35M35 groups of the first generation (z=-1.06, p=0.288).

The larval production rate decreased with each generation among the medusae reared in medium salinity, but increased with each generation among the medusae reared in low salinity (**Fig. 3.2 E, F**), due to the significant interaction of the salinity experienced by the medusae and the generation of the medusae (**Table 3.4**).

Table 3.4 Sexual reproduction rate. Results of the most parsimonious model, which was fitted assuming a negative binomial distribution. The highest applied salinities were excluded from this analysis (see statistical methods). The reference was set as P35M35G1 (hydroid colony reared at salinity 35, medusae reared at salinity 35, first generation).

	coef	exp (coef)	SE	z	Pr(>|z|)
(Intercept)	-2.3861	0.0920	0.1325	-18.01	<0.001
Polyp (Salinity = 25)	-0.4489	0.6383	0.1109	-4.05	<0.001
Medusa (Salinity = 25)	-1.9134	0.1476	0.2084	-9.18	<0.001
Generation (G2)	-0.0449	0.9561	0.1707	-0.26	0.793
Generation (G3)	-0.0949	0.9095	0.1777	-0.53	0.593
Medusa x Generation (Salinity = 25, G2)	0.6309	1.8793	0.2840	2.22	0.026
Medusa x Generation (Salinity = 25, G3)	1.1107	3.0365	0.2813	3.95	<0.001

3.4 Age-specific reproduction rates

I found that the three generations of medusae maintained in low salinity (**Fig. 3.3A**) had qualitatively very similar age-specific patterns of asexual reproductive rates (medusae budding rates) that differed only in the magnitude of the first peak (the highest peak was in the first generation; lower in the second generation and negligible in the third generation). After the peak in bud production, medusa budding continued until the end of life. This was not the case for larval production, which ceased long before the end of life (**Fig. 3.3C**).

In medium salinity (35), both medusae budding and larval production rates were highest at the beginning of life and decreased later in life **(Fig. 3.3 B and D)**. The initial peak in larval production was two times higher than that of maximal medusae budding. In low salinity (25), the initial peak in reproductive rates decreased with each generation, but the production of medusa buds (medusae budding) continued for the rest of each individual's life. The peak in larval production was reached at roughly the same age in all generations. While there was no clear maximum or distinct trend in sexual reproductive rates, it is worth noting that sexual reproduction ceased long before asexual reproduction **(Fig. 3.3D)**.

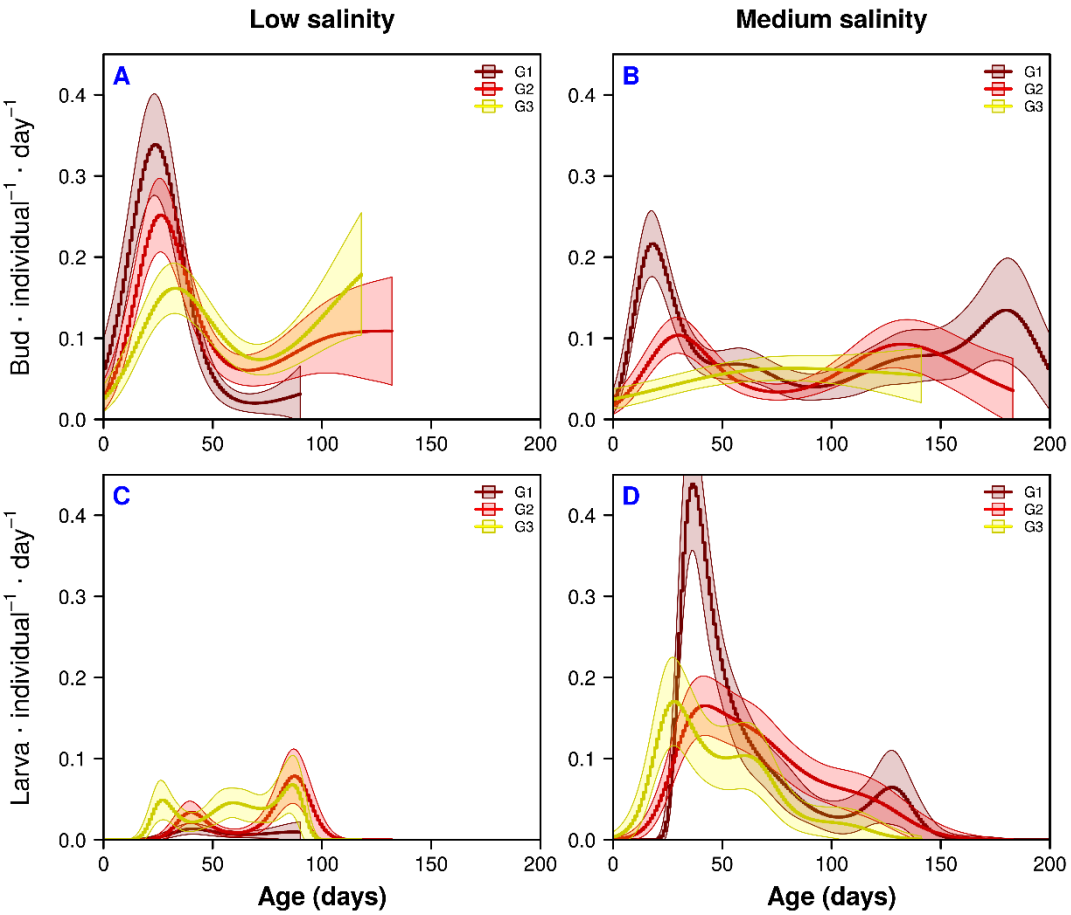

Figure 3.3 Age-specific asexual and sexual reproductive rates for the medusae maintained in low polyp/medusa salinity (panels A and C), and medium polyp/medusa salinity (panels B and D). The dashed lines mark the first maximum age of releasing either buds or larvae. G1, G2, and G3 are medusae from subsequent generations (first, second and third).

3.5 Survival

After an initial phase of rapid asexual and sexual reproduction, the medusae continued to produce buds as they gradually deteriorated. At the end of life, the medusae became deformed and turned brown (see supplementary materials, **Fig. A3**). Based on my observations, I noticed three patterns of medusae deterioration preceding death: 1) the medusa started shrinking and turning brown, and eventually dissolved or decomposed; 2) the medusa became smaller than the bud, and eventually detached from a bud in a terminal state; and 3) the medusa became smaller than the bud, and was eventually fully absorbed by a larger bud. The medusae that were

fully dissolved after a period of aging were considered dead, whereas the medusae absorbed by a bigger bud were treated as lost from the study.

Salinity experienced by hydroid colonies had no effect on the survival of medusae (supplementary materials, **Table A1**), but salinity experienced by medusae significantly affected their survival. The highest survival was observed in medusae reared in medium salinity, whereas the lowest survival was observed in medusae reared in the highest salinity (**Fig. 3.1 C**). The differences in survival among salinity treatments were significant in each generation (first three rows of **Table 3.5**). The effect of generation was analyzed separately for low and medium salinities. The medusae reared in high salinity were not included in the analysis due to the lack of a second and a third generation. I observed a significant effect of generation on the survival of medusae reared at low salinity, but not at medium salinity (last two rows of **Table 3.5**). The third generation of medusae reared in low salinity had slightly lower survival rates than those from other generations.

Table 3.5 Comparison of the survival distributions for the different generations and salinities experienced by the medusae; Two methods were used: log-rank and Gehan-Breslow. To avoid bias caused by multiple comparisons, the p-values were adjusted using Holm's method. M25, M35, and M45 are medusae maintained in salinities 25, 35, and 45 respectively. G1, G2, G3 are subsequent generations of medusae.

Group	Compared sub-groups	df	Log-rank stat	Log-rank p	Holm's Log-rank p	Gehan-Breslow stat	Gehan-Breslow p	Holm's Gehan-Breslow p
G1	M25, M35, and M45	2	88.87	0.0000	0.0000	110.99	0.0000	0.0000
G2	M25 and M35	1	5.65	0.0000	0.0000	6.46	0.0000	0.0000
G3	M25 and M35	1	3.32	0.0009	0.0027	3.31	0.0009	0.0027
M25	G1, G2 and G3	2	9.92	0.0070	0.0140	8.85	0.0120	0.0240
M35	G1, G2 and G3	2	3.61	0.1646	0.1646	3.17	0.2052	0.2052

3.6 Sizes of one-day old primary medusae

The log of the area of primary medusae (medusae of the first generation that were released by hydranths) was the largest in the P35M35 group (reference variable in regression analysis, **Table 3.6**); however, not all of my results were significant. The area of medusae was significantly lower in the highest salinity (17% less than among the reference group) and in the second generation (7% less in the lowest salinity than among the reference group).

Table 3.6 Effects of salinity and the effects of generation on the log of the area of one-day-old medusae (area was measured in µm2). The reference was set as P35M35G1 (hydroid colony reared at salinity 35, medusae reared at salinity 35, first generation).

	coef	exp (coef)	SE	t	Pr(>|t|)
(Intercept)	-0.6826	0.5053	0.0243	219.19	0.0000
Salinity (P25M25)	-0.0227	0.9776	0.0264	-0.86	0.3910
Salinity (P45M45)	-0.1890	0.8278	0.0613	-3.08	0.0024
Generation (G2)	-0.0772	0.9257	0.0305	-2.53	0.0121
Generation (G3)	-0.0472	0.9539	0.0335	-1.41	0.1605

4 Effects of temperature on reproduction and survival of medusae in two clones of *Eleutheria dichotoma* – (Results for Experiment II)

4.1 Sub-experiment 1: Effects of low (12°C) and medium temperature (23°C) on reproduction and survival of medusae

4.1.1 Effects of temperature on age at first asexual and sexual reproduction

Temperature influenced the age when the first medusa bud was released by a medusa (first asexual reproduction). Importantly, temperature of 12°C experienced by hydroid colony accelerated first asexual reproduction of primary medusae (first generation of medusae) (**Table 4.1, Fig. 4.1** upper panel). This effect was independent on temperature experienced by medusae, however it diminished in next generations. Opposite to this, temperature of 12°C experienced by medusae postponed their first asexual reproduction. This effect was prominent in all generations. The effect of generation was significant in treatments where rearing temperatures were changed between hydroid colony and medusae. For each of the four combination of treatments larger differences were observed between generations G1 and G2 than between G2 and G3. Particularly, the first generation of P12 M23 (hydroid colony maintained at temperature of 12°C and medusae maintained at temperature of 23°C) matured earlier, and the first generation of P23C M12 (hydroid colony maintained at temperature of 23°C and medusae maintained at temperature of 12°C) matured later, which indicated clear effect of the temperature experienced by hydroid colony.

The temperature experienced by medusae had strong effect on sexual maturity and production of planula larvae. Although medusae reared at 12°C typically reached sexual maturity as embryos were observed in brood pouches, most of the embryos did not complete development to larva stage. Low number of larvae released at these conditions were dead (round-formed, pale, and non-moving) and only in few cases larvae were alive and motile.

Medusae reared in 23°C matured earlier than medusae reared in 12°C. There was significant effect of temperature experienced by polyps on sexual maturity of medusae in the first generation (**Table 4.1, Fig. 4.1** middle panel). Medusae that had detached from hydroid colony at 12°C and were transferred to 23°C initiated larvae production earlier than medusae released by hydroid colony at 23°C and reared further in these conditions. Interestingly, in treatment P12M12 there was strong delay in maturity with consecutive generations. There was also small, but still significant delay in maturity in third generation of P12M23 and P23M23 treatments.

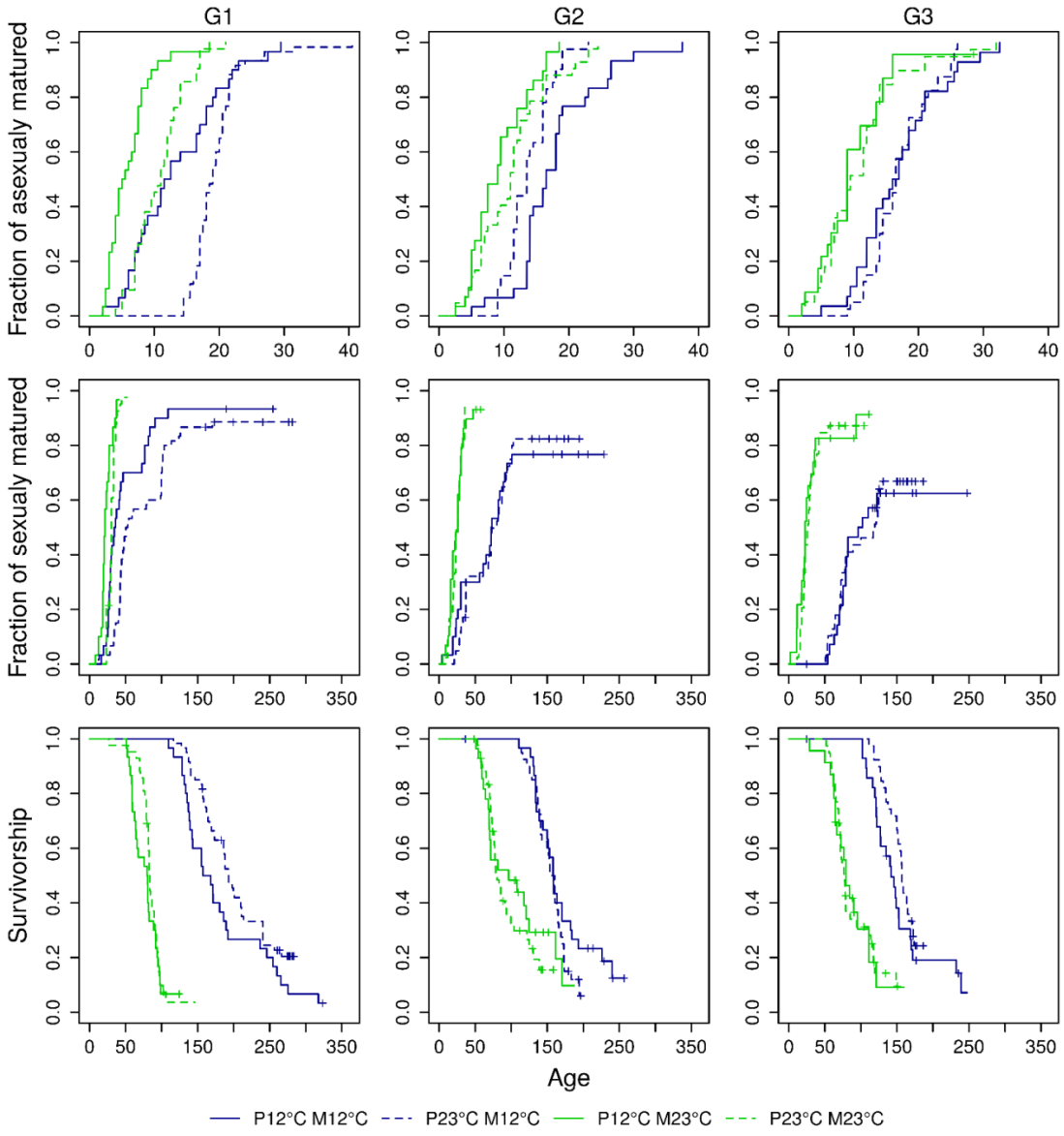

Figure 4.1 Asexual maturity, sexual maturity, and survivorship in three generations of medusae Eleutheria dichotoma maintained in different temperatures. The first two rows represent the Kaplan-Meier estimators of asexual and sexual maturity (inverse of KM). The third row represents the survival of the medusae in the different temperatures. The medusae that detached from hydroid colonies at one temperature were transferred to different temperature conditions. The line patterns represent the different temperatures experienced by the hydroid colonies; the colours represent the different temperatures experienced by the medusae; and the columns represent the different generations of the medusae. Age is measured in days. Medusae from regime P23M23 were released by hydroid colony at temperature 23°C and were maintained in the same temperature. Medusae from regime P12M23 were released by hydroid colonies at temperature 12°C and were transferred to temperature 23°C. The other regimes should be read analogically.

Table 4.1 Asexual maturity of three generations (G) of medusae Eleutheria dichotoma at different combinations of temperature experienced by polyps (P) and medusae (M). The non-parametric comparisons (Log-rank and Gehan-Breslow tests) were corrected by the Holm's method.

	Comparison	df	Log-rank stat	Log-rank p	Holm's Log-rank p	Gehan-Breslow stat	Gehan-Breslow p	Holm's Gehan-Breslow p	
Effect of temp. experienced by polyps	P12°C vs. P23°C for M12°C, G1	1	-2.27	0.0231	0.1848	-4.11	0.0000	0.0000	***
	P12°C vs. P23°C for M23°C, G1	1	-3.60	0.0003	0.0042	-4.78	0.0000	0.0000	***
	P12°C vs. P23°C for M12°C, G2	1	3.30	0.0010	0.0130	3.18	0.0015	0.0135	*
	P12°C vs. P23°C for M23°C, G2	1	-1.52	0.1275	0.8925	-1.40	0.1615	0.6460	
	P12°C vs. P23°C for M12°C, G3	1	0.53	0.5940	1.0000	-0.41	0.6805	1.0000	
	P12°C vs. P23°C for M23°C, G3	1	-0.35	0.7291	1.0000	-0.51	0.6092	1.0000	
	P12°C vs. P23°C for M12°C, pooled generations	1	-0.02	0.9812	1.0000	-1.83	0.0676	0.3380	
	P12°C vs. P23°C for M23°C, pooled generations	1	-2.98	0.0029	0.0319	-3.87	0.0001	0.0011	***
Effect of temp. experienced by medusae	M12°C vs. M23°C for P12°C, G1	1	4.03	0.0001	0.0015	4.41	0.0000	0.0000	***
	M12°C vs. M23°C for P23°C, G1	1	6.00	0.0000	0.0000	7.84	0.0000	0.0000	***
	M12°C vs. M23°C for P12°C, G2	1	4.24	0.0000	0.0000	4.87	0.0000	0.0000	***
	M12°C vs. M23°C for P23°C, G2	1	1.21	0.2281	1.0000	2.87	0.0041	0.0287	*
	M12°C vs. M23°C for P12°C, G3	1	2.98	0.0029	0.0319	3.86	0.0001	0.0011	***
	M12°C vs. M23°C for P23°C, G3	1	3.25	0.0012	0.0144	5.10	0.0000	0.0000	***
	M12°C vs. M23°C for P12°C, pooled generations	1	6.30	0.0000	0.0000	7.40	0.0000	0.0000	***
	M12°C vs. M23°C for P23°C, pooled generations	1	6.71	0.0000	0.0000	9.28	0.0000	0.0000	***
Effect of medusae generation	G1 vs. G2 vs. G3 for P12°C, M12°C	2	3.62	0.1638	0.9828	6.62	0.0365	0.2190	
	G1 vs. G2 vs. G3 for P12°C, M23°C	2	24.72	0.0000	0.0000	43.64	0.0000	0.0000	***
	G1 vs. G2 vs. G3 for P23°C, M12°C	2	8.40	0.0150	0.1350	12.26	0.0022	0.0176	*
	G1 vs. G2 vs. G3 for P23°C, M23°C	2	0.24	0.8861	1.0000	0.36	0.8347	1.0000	

Table 4.2 Sexual maturity of three generations (G) of medusae Eleutheria dichotoma at different combinations of temperature experienced by polyps (P) and medusae (M). The non-parametric comparisons (Log-rank and Gehan-Breslow tests) were corrected by the Holm's method.

	Comparison	df	Log-rank stat	Log-rank p	Holm's Log-rank p	Gehan-Breslow stat	Gehan-Breslow p	Holm's Gehan-Breslow p	
Effect of temp. experienced by polyps	P12°C vs. P23°C for M12°C, G1	1	-2.49	0.0128	0.1152	-3.42	0.0006	0.0054	***
	P12°C vs. P23°C for M23°C, G1	1	-2.99	0.0028	0.0280	-4.35	0.0000	0.0000	***
	P12°C vs. P23°C for M12°C, G2	1	0.02	0.9815	1.0000	-0.48	0.6315	1.0000	
	P12°C vs. P23°C for M23°C, G2	1	0.57	0.5721	1.0000	-0.38	0.7034	1.0000	
	P12°C vs. P23°C for M12°C, G3	1	0.10	0.9209	1.0000	0.12	0.9006	1.0000	
	P12°C vs. P23°C for M23°C, G3	1	-0.44	0.6579	1.0000	-0.93	0.3537	1.0000	
	P12°C vs. P23°C for M12°C, pooled generations	1	-0.62	0.5328	1.0000	-1.43	0.1531	0.9186	
	P12°C vs. P23°C for M23°C, pooled generations	1	-1.61	0.1078	0.7546	-3.11	0.0019	0.0133	*
Effect of temp. experienced by medusae	M12°C vs. M23°C for P12°C, G1	1	4.11	0.0000	0.0000	4.49	0.0000	0.0000	***
	M12°C vs. M23°C for P23°C, G1	1	5.80	0.0000	0.0000	6.91	0.0000	0.0000	***
	M12°C vs. M23°C for P12°C, G2	1	4.06	0.0000	0.0000	4.28	0.0000	0.0000	***
	M12°C vs. M23°C for P23°C, G2	1	6.08	0.0000	0.0000	6.77	0.0000	0.0000	***
	M12°C vs. M23°C for P12°C, G3	1	3.97	0.0001	0.0013	4.86	0.0000	0.0000	***
	M12°C vs. M23°C for P23°C, G3	1	5.34	0.0000	0.0000	6.45	0.0000	0.0000	***
	M12°C vs. M23°C for P12°C, pooled generations	1	6.90	0.0000	0.0000	7.92	0.0000	0.0000	***
	M12°C vs. M23°C for P23°C, pooled generations	1	9.69	0.0000	0.0000	11.51	0.0000	0.0000	***
Effect of medusae generation	G1 vs. G2 vs. G3 for P12°C, M12°C	2	16.43	0.0003	0.0036	20.64	0.0000	0.0000	***
	G1 vs. G2 vs. G3 for P12°C, M23°C	2	12.59	0.0018	0.0198	15.77	0.0004	0.0040	***
	G1 vs. G2 vs. G3 for P23°C, M12°C	2	2.17	0.3383	1.0000	0.26	0.8799	1.0000	
	G1 vs. G2 vs. G3 for P23°C, M23°C	2	8.44	0.0147	0.1176	14.10	0.0009	0.0072	***

4.1.2 Asexual reproduction rates (medusae budding rates)

Both temperature experienced by polyps and temperature experienced by medusae had strong effect on medusae budding rates (**Table 4.3, Fig. 4.2** left panel). Medusae reared at 23°C produced buds with 1.3 times higher rates than medusae reared at 12°C. Similarly, medusae that detached from hydroid colonies at 23°C produced buds with 1.16 times higher rates than medusae that detached from hydroid colonies at 12°C. Budding rates decreased with generation by medusae maintained at 23°C (**Table 4.3**).

Table 4.3 Asexual reproduction of medusae E. dichotoma. Different models were fitted to the reproduction data. Reproduction rate for asexual reproduction was modelled as a function of temperature experienced by polyp, temperature experienced by medusae and generation.

	exp(Coef)	Coef	SE	z value	Pr(>|z|)
(Intercept)	0.0804	-2.5209	0.0450	-55.99	0.0000
Polyps (23°C)	1.3449	0.2963	0.0330	8.98	0.0000
Medusae (23°C)	1.1562	0.1452	0.0524	2.77	0.0056
Generation (G2)	0.8998	-0.1056	0.0619	-1.71	0.0881
Generation (G3)	0.9123	-0.0917	0.0659	-1.39	0.1637
Polyps (23°C) : generation (G2)	0.7758	-0.2539	0.0797	-3.18	0.0015
Polyps (23°C) : generation (G3)	0.9840	-0.0161	0.0816	-0.20	0.8434

4.1.3 Sexual reproduction rates (larval production rates)

Larval production rate was significantly lower at temperature 12°C than in other regimes. Medusae reached sexual maturity, as the embryos were observed, but the development of embryos was incomplete. Consequently, a low number of these medusae released non-motile and round-shaped larvae, which were considered as dead. Larval production rate at temperature 23°C significantly decreased at 3rd generation (**Fig. 4.2**, significant interaction in **Table 4.4**). There was no effect of low temperature experienced by polyps on larval production rate of medusae.

Table 4.4 Sexual reproduction of medusae E. dichotoma. Different models were fitted to the reproduction data. Sexual reproduction rate was modelled as a function of temperature experienced by polyp, temperature experienced by medusae and generation.

	exp(Coef)	Coef	SE	z value	Pr(>|z|)
(Intercept)	0.0157	-4.1527	0.1202	-34.54	0.0000
Polyps (23°C)	0.9581	-0.0428	0.0895	-0.48	0.6323
Medusae (23°C)	16.5380	2.8057	0.1424	19.70	0.0000
Generation (G2)	1.1847	0.1695	0.1572	1.08	0.2809
Generation (G3)	1.0044	0.0044	0.1629	0.03	0.9784
Medusae (23°C) : generation (G2)	0.7427	-0.2974	0.2089	-1.42	0.1546
Medusae (23°C) : generation (G3)	0.4842	-0.7252	0.2185	-3.32	0.0009

4.1.4 Survival

Temperature experienced by medusae significantly affected their survival in all treatments (**Table 4.5**). Low temperature (12°C) prolonged medusae lifespan in all generations (**Fig. 4.1**). There was no effect of generation within treatments, except from P12M23 group, where the first generation of medusae lived slightly longer than others. There was no effect of temperature experienced by hydroid colonies on survival of medusae.

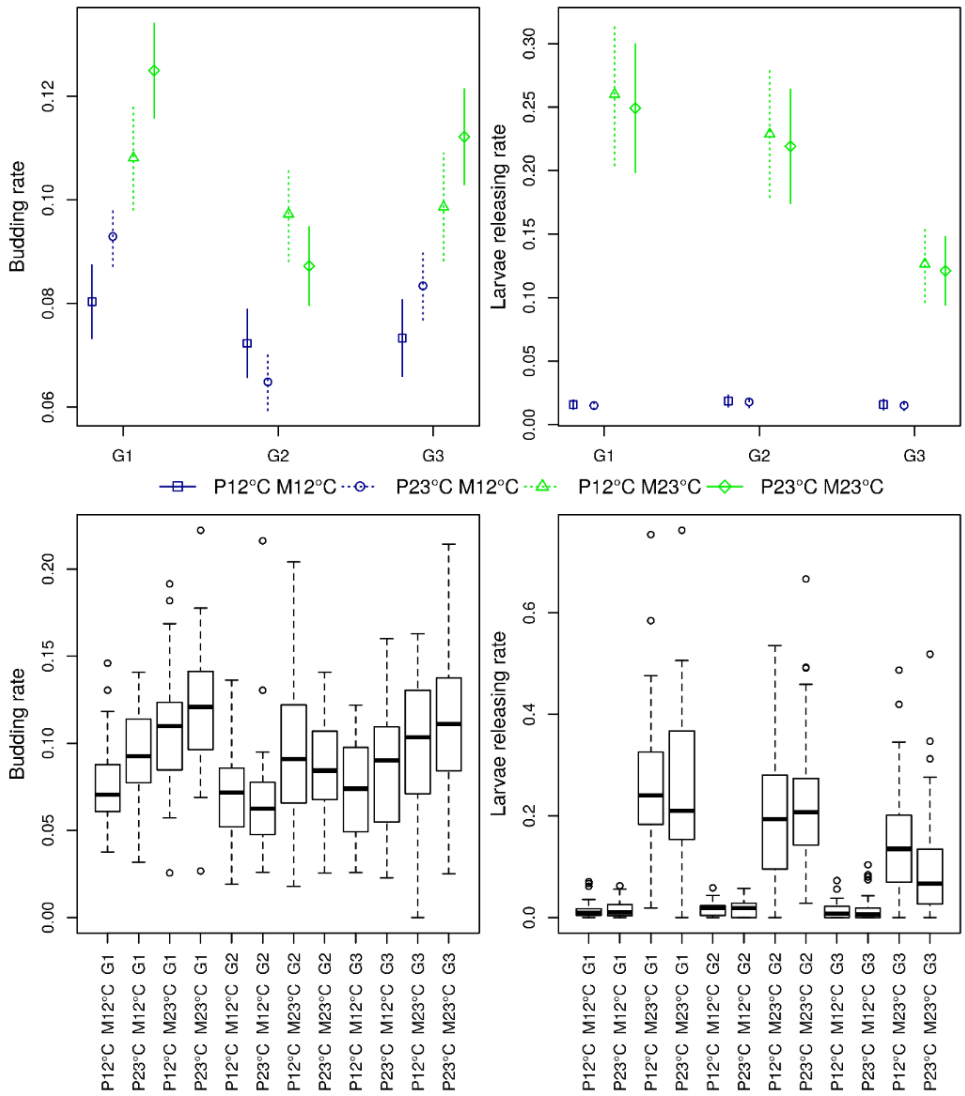

Figure 4.2 Asexual reproduction rate (medusa budding rate) and sexual reproduction (larval production rate) in three generations (G1 – G3) of medusae maintained in different temperatures. Upper panels – prediction of fitted models, lower panels – underlying data summarized by box-plots.

Table 4.5 Comparison of the survival distributions for the different temperatures experienced by hydroid colonies (P12, P23) and different generations (G1, G2, G3) and temperatures experienced by the medusae (M12, M23); Two methods were used: log-rank and Gehan-Breslow. To avoid bias caused by multiple comparisons, the p-values were adjusted using Holm's method.

	Comparison	df	Log-rank stat	Log-rank p	Holm's Log-rank p	Gehan-Breslow stat	Gehan-Breslow p	Holm's Gehan-Breslow p	
Effect of temp. exp. by colonies	P12°C vs. P23°C for M12°C, G1	1	-2.03	0.0421	0.4210	-2.46	0.0140	0.1540	
	P12°C vs. P23°C for M23°C, G1	1	-1.03	0.3019	1.0000	-1.90	0.0573	0.4584	
	P12°C vs. P23°C for M12°C, G2	1	1.21	0.2258	1.0000	0.64	0.5196	1.0000	
	P12°C vs. P23°C for M23°C, G2	1	0.69	0.4893	1.0000	0.04	0.9647	1.0000	
	P12°C vs. P23°C for M12°C, G3	1	-1.41	0.1597	1.0000	-2.05	0.0399	0.3591	
	P12°C vs. P23°C for M23°C, G3	1	0.16	0.8710	1.0000	-0.02	0.9879	1.0000	
	P12°C vs. P23°C for M12°C, pooled generations	1	-1.86	0.0630	0.5670	-2.44	0.0149	0.1540	
	P12°C vs. P23°C for M23°C, pooled generations	1	0.21	0.8346	1.0000	-0.99	0.3198	1.0000	
Effect of temp. exp. by medusae	M12°C vs. M23°C for P12°C, G1	1	5.24	0.0000	0.0000	6.43	0.0000	0.0000	***
	M12°C vs. M23°C for P23°C, G1	1	6.59	0.0000	0.0000	8.29	0.0000	0.0000	***
	M12°C vs. M23°C for P12°C, G2	1	3.15	0.0016	0.0192	4.09	0.0000	0.0000	***
	M12°C vs. M23°C for P23°C, G2	1	4.41	0.0000	0.0000	5.75	0.0000	0.0000	***
	M12°C vs. M23°C for P12°C, G3	1	3.64	0.0003	0.0039	4.61	0.0000	0.0000	***
	M12°C vs. M23°C for P23°C, G3	1	5.28	0.0000	0.0000	6.30	0.0000	0.0000	***
	M12°C vs. M23°C for P12°C, pooled generations	1	6.86	0.0000	0.0000	8.82	0.0000	0.0000	***
	M12°C vs. M23°C for P23°C, pooled generations	1	9.56	0.0000	0.0000	11.99	0.0000	0.0000	***
Effect of generation	G1 vs. G2 vs. G3 for P12°C, M12°C	2	4.27	0.1184	0.9472	5.55	0.0624	0.4584	
	G1 vs. G2 vs. G3 for P12°C, M23°C	2	22.27	0.0000	0.0000	23.40	0.0000	0.0000	***
	G1 vs. G2 vs. G3 for P23°C, M12°C	2	8.19	0.0167	0.1837	3.84	0.1466	0.8796	
	G1 vs. G2 vs. G3 for P23°C, M23°C	2	1.88	0.3913	1.0000	2.08	0.3529	1.0000	

4.1.5 Size of medusae at birth and at first sexual reproduction

The temperature had significant effect on the (log of) area of medusae at birth and at first sexual reproduction. The area of one-day old medusae that had detached from hydroid colonies reared at low temperature (12°C) was roughly two times bigger than medusae reared in medium temperature (23°C) (**Fig. 4.3** left panel; **Table 4.6 a, Fig. A7,** supplementary materials **Fig. A5, A6**). The (log of) area at sexual maturity was the highest for medusae continuously reared at 12°C (reference group, P12/M12, **Fig. 4.3** right panel; **Table 4.6 b**). Medusae reared at these conditions rarely released larvae but embryos were observed in umbrella for some period. The beginning of this period was considered as the onset of sexual maturity. Medusae that detached from polyps at 12°C and were transferred to 23°C, similarly as medusae that were continuously reared at 23°C had roughly 1.8 times smaller area at the day of maturity than the reference group (P12/M12). The area of medusae that had detached from polyps at 23°C and were transferred to 12°C were 1.2 times smaller at the age of maturity than the reference group.

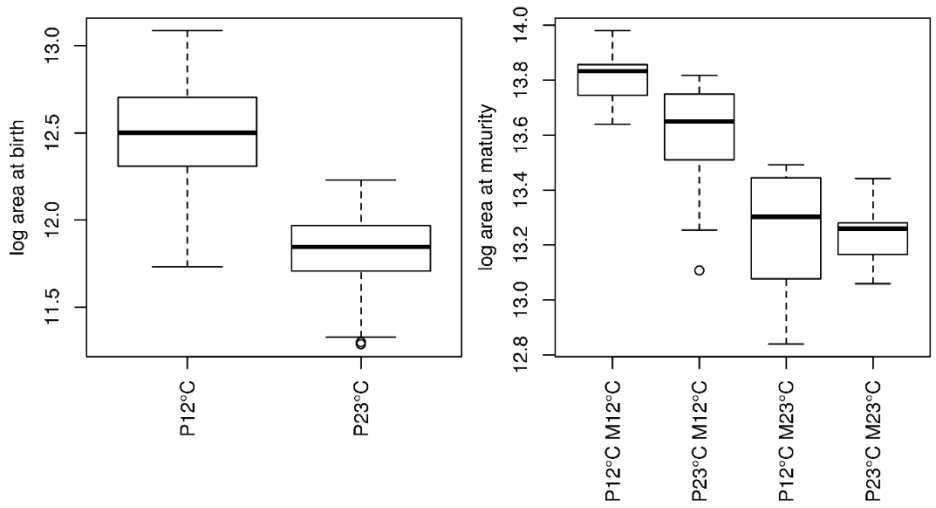

*Figure 4.3 Logarithm of area of medusae Eleutheria dichotoma [µm2]. **A.** Area of medusae at the day of detachment from hydroid colonies maintained at temperature of 12°C or 23°C; **B.** Area of medusae at the first sexual maturity (when embryos were observed in brood pouch or first larva was released).*

*Table 4.6 Summary table for models fitted to the log of area. Log area was modelled as a function of temperature experienced by hydroid colonies, temperature experienced by medusae and generation of medusae for (**a**) one-day old medusae and (**b**) medusae at sexual reproduction*

	exp(Coef)	Coef	SE	t value	Pr(>|t|)
(a) one-day old medusae					
(Intercept)	269519.7052	12.5044	0.0371	337.45	0.000
Polyp temp. (23°C)	0.5072	-0.6788	0.0440	-15.43	0.000
(b) medusae at sexual reproduction					
(Intercept)	995465.8727	13.8110	0.0314	440.28	0.000
Polyp temp. (23°C)	0.8155	-0.2039	0.0378	-5.40	0.000
Medusa temp. (23°C)	0.5641	-0.5726	0.0449	-12.76	0.000
Polyp temp. (23°C) : Medusa temp. (23°C)	1.2247	0.2027	0.0568	3.57	0.001

4.2 Sub-experiment 2: Effects of high (28°C) and medium temperature (23°C) on reproduction and survival of the first generation of medusae (primary medusae)

4.2.1 Effects of temperature on age at first asexual and sexual reproduction

Temperature experienced by medusae significantly affected the age of their first asexual reproduction (**Table 5a**). Medusae reared at 28°C delayed onset of budding and many of them never produced buds. These medusae never reproduced sexually. Temperature experienced by polyps had no effect neither on asexual nor sexual maturity of medusae in the studied first generation **(Fig. 8, Table 5a** and **b** respectively**).**

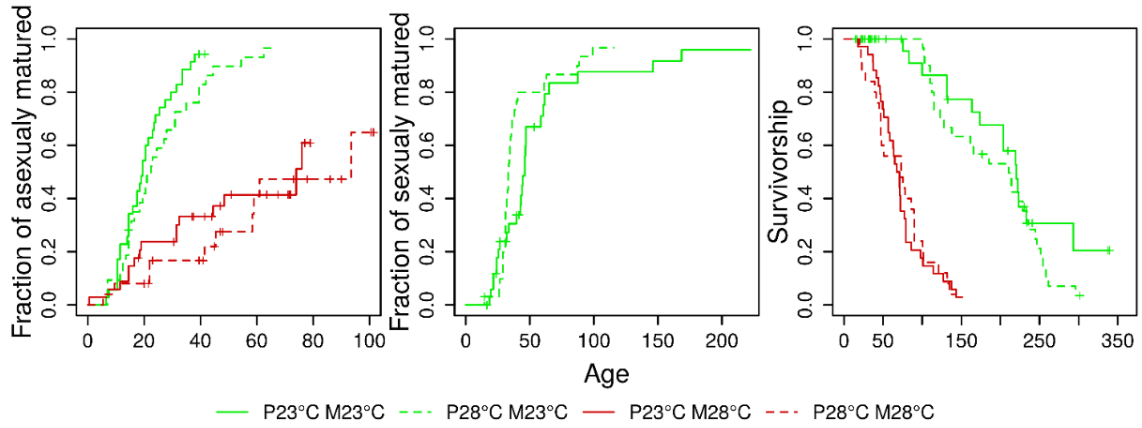

Figure 4.4 Kaplan-Meier estimators (or inverse of it) for asexual maturity, sexual maturity, and survivorship of first generation of medusae Eleutheria dichotoma maintained in different temperatures experienced by polyp (P) and medusa (M). Asexual maturity was measured as age of releasing first bud, and sexual maturity was measured as age of the first observation of developing embryos or release of the first larva.

Table 4.7 Asexual maturity of hydromedusae Eleutheria dichotoma at different combinations of temperature experienced by polyps and medusae. The non-parametric comparisons (Log-rank and Gehan-Breslow tests) were corrected by the Holm's method

	Comparison	df	Log-rank stat	Log-rank p	Holm's Log-rank p	Gehan-Breslow stat	Gehan-Breslow p	Holm's Gehan-Breslow p	
(a) Asexual maturity									
Temp. experienced by polyps	G1 P23°C vs. P28°C for M23°C	1	1.47	0.1427	0.2854	1.15	0.2509	0.5018	
	G1 P23°C vs. P28°C for M28°C	1	0.71	0.4764	0.4764	0.87	0.3865	0.5018	
Temp. experienced by medusae	G1 M23°C vs. M28°C for P23°C	1	4.69	0.0000	0.0000	4.15	0.0000	0.0000	***
	G1 M23°C vs. M28°C for P28°C	1	4.84	0.0000	0.0000	4.21	0.0000	0.0000	***
(b) Sexual maturity									
	P23°C vs. P28°C for M23°C	1	1.91	0.0558	0.0558	1.63	0.1039	0.1039	.

4.2.2 Asexual reproduction rates (medusae budding) and sexual reproduction rates (larval production)

High temperature (28°C) negatively affected medusae budding rates. Medusae reared at 28°C produced almost eight time less buds than in other regimes (**Table 7a),** and no larvae at all (**Fig. 9** right panel). There was no effect of temperature of 28°C experienced by hydroid colonies on budding rates of medusae transferred to 23°C (**Fig. 9**, **Table 7a** and **b**). However, temperature 28°C experienced by hydroid colonies increased roughly 1.7 times larval production rates of medusae maintained at 23°C (**Table 7b**).

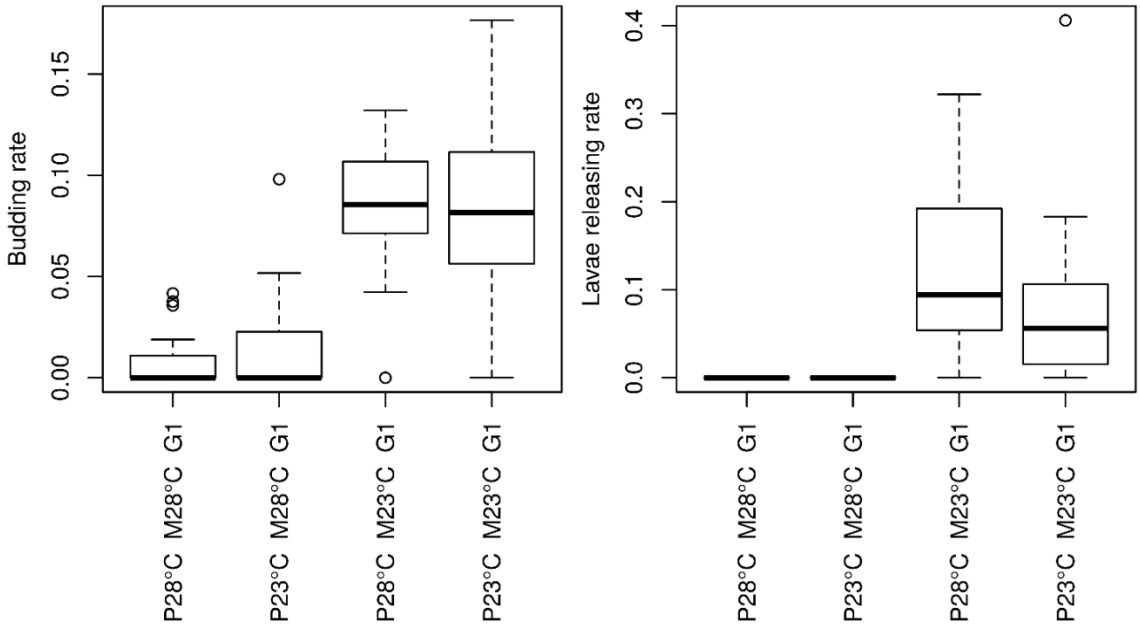

Figure 4.5 The rates of bud and larva production in first generation of hydromedusae Eleutheria dichotoma maintained at different temperatures. Medusae from regime P23M23 were released by hydroid colony at temperature 23C and were maintained in the same temperature. Medusae from regime P28M23 were released by hydroid colony at temperature 28C and were transferred to temperature 23C. The other regimes should be read analogically.

*Table 4.8 Summary table for models fitted to the reproduction data. Reproduction rate was modelled as a function of temperature experienced by polyp and medusae for (**a**) asexual reproduction and (**b**) sexual reproduction*

	exp(Coef)	Coef	SE	z value	Pr(>|z|)
(a) asexual reproduction					
(Intercept)	0.0115	-4.4653	0.1488	-30.01	0.000
Temp. exp. by polyps (23°C)	1.0333	0.0327	0.0756	0.43	0.665
Temp. exp. by medusae (23°C)	7.9090	2.0680	0.1476	14.01	0.000
(b) sexual reproduction					
(Intercept)	0.1308	-2.0339	0.1417	-14.35	0.0000
Polyp temp. (23°C)	0.5784	-0.5476	0.2035	-2.69	0.0071

4.2.3 Survival

Lifespan of medusae was significantly shortened at temperature of 28°C. There was no effect of temperature 28°C experienced by polyps on survival of medusae maintained at 23°C (**Table 4.9, Fig. 4.4**).

Table 4.9 Survival of hydromedusae Eleutheria dichotoma at different combinations of temperature experienced by polyps and medusae. The non-parametric comparisons (Log-rank and Gehan-Breslow tests) were corrected by the Holm's method.

	Comparison	df	Log-rank stat	Log-rank p	Holm's Log-rank p	Gehan-Breslow stat	Gehan-Breslow p	Holm's Gehan-Breslow p	
Effect of temperature experienced by polyps	G1: P23°C vs. P28°C for M23°C	1	-1.06	0.2912	0.5824	-0.70	0.4857	0.9714	
	G1: P23°C vs. P28°C for M28°C	1	0.03	0.9800	0.9800	0.05	0.9633	0.9714	
Effect of temperature experienced by medusae	G1: M23°C vs. M28°C for P23°C	1	-5.66	0.0000	0.0000	-5.80	0.0000	0.0000	***
	G1: M23°C vs. M28°C for P28°C	1	-4.67	0.0000	0.0000	-5.69	0.0000	0.0000	***

4.2.4 Size of one-day old medusae (primary medusae) at birth and at first sexual reproduction

The log of area of one-day old medusae that had detached from hydroid colonies at temperature 28°C was roughly 1.2 times smaller than log of area of medusae that had detached from hydroid colonies at temperature 23°C **(Fig. 7, Table 4.10 a).** Medusae reared at high temperature never matured sexually. High temperature induced morphological changes. Medusae produced many tentacles and their umbrella was deformed (supplementary materials Fig. A4). Medusae that had detached from hydroid colonies at temperature 28°C and were later transferred to medium temperature 23°C were roughly 1.1 times smaller (close to significance, **Table 4.10 b**) at the age of maturity than medusae that were constantly reared at 23°C.

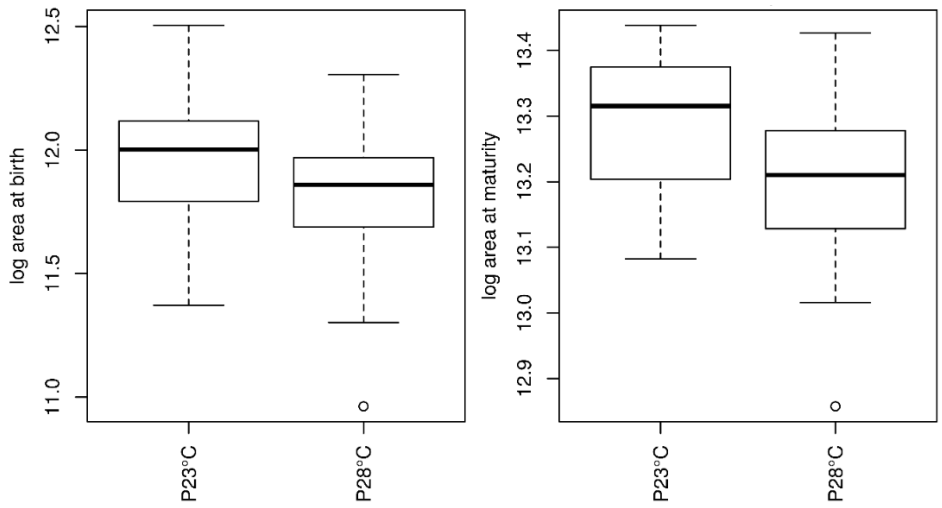

Figure 4.6 Size of medusae Eleutheria dichotoma at birth (one-day old) and at the first sexual reproduction at combinations of medium and high temperatures (23°C and 28°C).

Table 4.10 Summary table for models fitted to the log of area. Log area as a function of temperature experienced by polyp for (a) one-day old medusae and (b) medusae at sexual reproduction

	exp(Coef)	Coef	SE	t value	Pr(>|t|)
(a) one-day old medusae					
(Intercept)	158338.0986	11.9725	0.0285	420.45	0.000
Polyp temp. (28°C)	0.8292	-0.1873	0.0546	-3.43	0.001
(b) medusae at sexual maturity					
(Intercept)	585796.9338	13.2807	0.0323	411.00	0.000
Polyp temp. (28°C)	0.9184	-0.0851	0.0404	-2.11	0.042

5 Effects of population density on reproduction and survival of medusae in hydrozoan *Eleutheria dichotoma* – (Results for Experiment III)

5.1 Patterns of survivorship and mortality

I found significant differences in the survivorships of the three density regimes (log-rank, χ^2= 253.4, p<0.0001, **Fig. 5.1,** left panel). The lowest survival rates were in the highest density conditions (25 individuals in a well, D25), while the highest survival rates were in the lowest density conditions (one individual per well, D1). These results were confirmed by the trend test (Gehan-Mantel test, Z=-14.66, p<0.0001). The empirical mortality rates were fitted well by a γ-Gompertz model (Vaupel et al. 1979; Missov et al. 2016), and were reflected by curves indicating an exponential rate of growth early in life, which then decelerated and reached a plateau at older ages (**Fig. 5.1,** right panel). The three curves were similar in the intercept parameter a (LRT, ratio=0.1224, p=0.9406, a=5.26 $\cdot$ 10^{-7}), but differed in the rate-of-aging parameter b (LRT, ratio=8.270, p=0.0160) and the heterogeneity parameter γ (LRT, ratio=11.04, p=0.0040). The population-specific heterogeneity decreased with population density (D1: γ =9.31, D10: γ =2.41, and D25: γ =1.28), whereas the rate of aging increased with population density (D1: b=0.139, D10: b=0.157, and D25: b=0.193). Qualitatively and quantitatively similar results were obtained when each population was fitted separately (**Fig. 5.1** right panel, dotted lines).

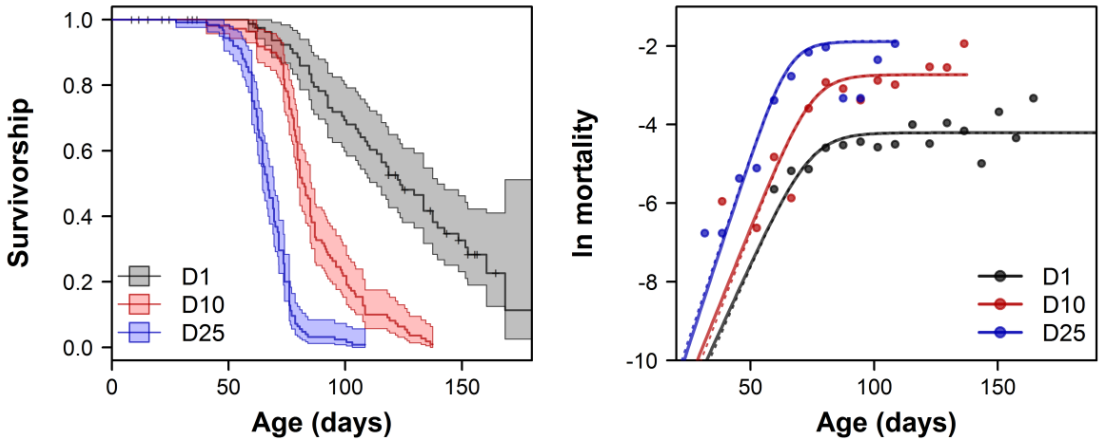

Figure 5.1 Survivorship of medusae and logarithmized mortality rates at different densities. The survivorships (left panel) were based on the Kaplan-Meier estimator of survival. Marker "+" represents censoring events (medusae lost or absorbed by a bigger bud). The empirical mortality rates (right panel) were fitted with a γ-Gompertz model of mortality using a maximum likelihood method. The solid lines represent the fit of the most parsimonious model that assumes a common a *parameter for all densities, whereas the dashed lines (which overlap considerably with the solid lines) represent each population that was fitted separately by this model. The empirical mortality rates are represented as dots that were calculated from seven-day aggregated data.*

5.2 Reproduction rate

The asexual reproduction rate decreased with density (GLM, Poisson errors, density as continuous predictor, χ^2=151.2, p<0.0001). The medusae that were maintained individually had the highest asexual reproduction rate, whereas the medusae that were reared in the highest density conditions had a rate that was 3.5 times lower (**Fig. 5.2** left panel). Furthermore, the variation in the reproduction rate between individual medusae was much lower in D10 and in D25 than in D1 (coefficient of variation, D1: 0.341, D10: 0.160, D25: 0.164). Density also influenced the sexual reproduction rate (GLM, Poisson errors, density as categorical predictor, χ^2=1047, p<0.0001), but there was no linear trend (**Fig. 5.2** right panel). I applied multiple comparisons of means (Tukey contrasts) as a post hoc to the fitted model. The highest rate of larvae production was observed in the medusae maintained in D10 (0.962 larvae per medusa per week). This rate was 1.577 times higher than in D1 (z=3.170, p=0.0042), and it was 1.637 times higher than in D25 (z=3.248, p=0.0033). There was no significant difference in the larvae

production rate between D1 and D25 ($z=0.0226$, $p=0.9722$). The age-specific asexual reproduction rate changed with age in all three regimes of density (**Fig. 5.3**). The reproduction rate of the medusae that were maintained individually displayed a clear pattern: the rate increased at the beginning of life, peaked roughly between the 20^{th} and the 30^{th} day of life, and decreased later in life. A similar pattern, but with a lower peak reproduction rate, was observed in regime D25. The initial peak in the reproduction rate was lowest in regime D10. For the medusae from all of the regimes, asexual reproduction continued over the whole life course, and even increased late in life. In all of the density regimes, the pattern of the age-specific sexual reproduction rate was similar to that of the asexual reproduction rate. The rate of reproduction increased at the beginning of life, reached a peak between the 30^{th} and the 40^{th} day, and then decreased with age. Sexual reproduction ended early and well before the end of the medusa's life. The medusae from regimes D10 and D25 tended to initiate sexual reproduction earlier than the medusae from D1.

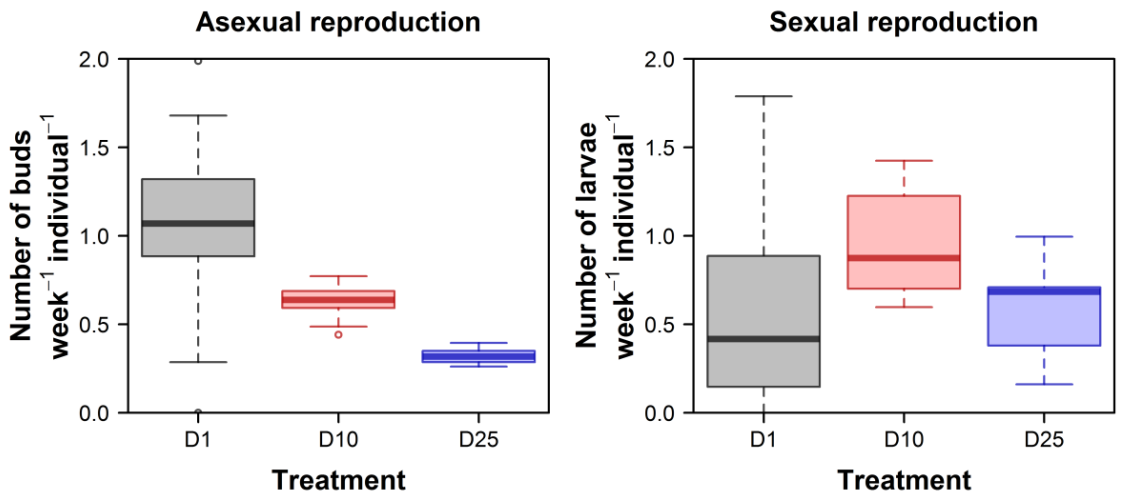

*Figure 5.2 Asexual and sexual reproduction of medusae maintained in different densities. The box plots represent reproduction rates counted as a sum of released buds divided by the sum of the lifespans of all of the individuals. All of the medusae were fed according to the same feeding regime (*Artemia ad libitum*).*

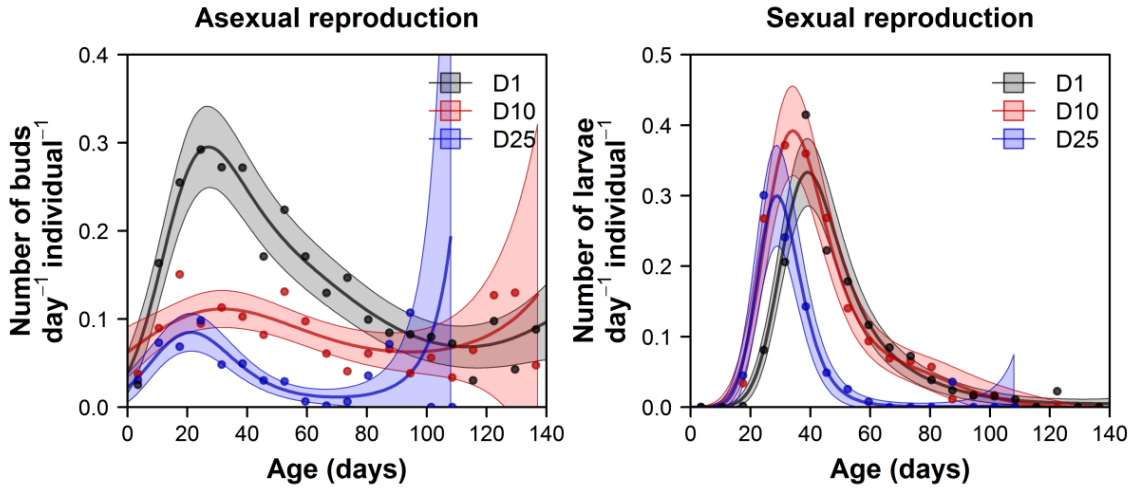

Figure 5.3 Age-specific asexual (A) and sexual (B) reproduction rates. The colors represent different regimes of density: gray (one medusa in a well), red (10 medusae in a well), and blue (25 medusae in a well). The increased asexual reproductive rate of D25 in the higher age classes might be the effect of decreased density in experimental wells due to deaths (dead individuals were not replaced).

5.3 Sizes of the medusae at birth and at sexual maturation

The analysis of variance (ANOVA) showed significant differences between the average area of medusa (eq. ii) at birth and the average area (eq. iii) at sexual maturity for each density ($F=453.5$, $p<0.0001$). The average area of the sexually mature medusae in the different densities was roughly 2.8 times bigger than the average area recorded at birth (**Fig. 5.4**, Tukey HSD, the area at birth compared with every density always gives $p<0.0001$). However, the average areas of the sexually mature medusae did not differ between the regimes (Tukey HSD, D1-D10: $p=0.7470$, D1-D25: $p=0.9942$, D10-D25: $p=0.6071$).

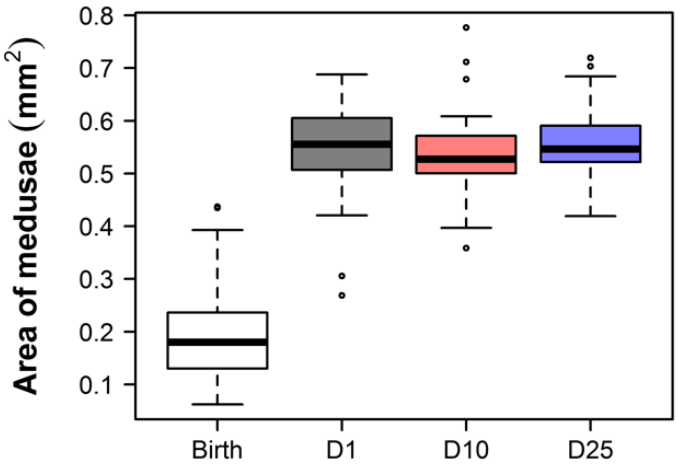

Figure 5.4 Area of the medusae at birth and at sexual maturation, reared in different densities. The area of the medusae was calculated according to Schierwater (1989) formulas.

5.4 Size of larvae

A multivariate analysis of covariance (MANCOVA, type III of sum of squares) showed that there was a significant effect of density on the length and the width of larvae ($F= 8.56$, $p=0.0002$). There was, however, no significant main effect of the day of the experiment ($F=0.08$, $p=0.9267$), and there was significant interaction between the density and the

length/width of the larvae (F=3.80, p=0.0233). The larvae released in densities 10 and 25 were smaller at day 50 of the experiment (when I started measuring their sizes), but their sizes increased with time, eventually reaching the sizes of the larvae produced by medusae reared individually. The sizes of the larvae released by the medusae that were individually maintained were roughly constant during the experiment (Fig. 5.5).

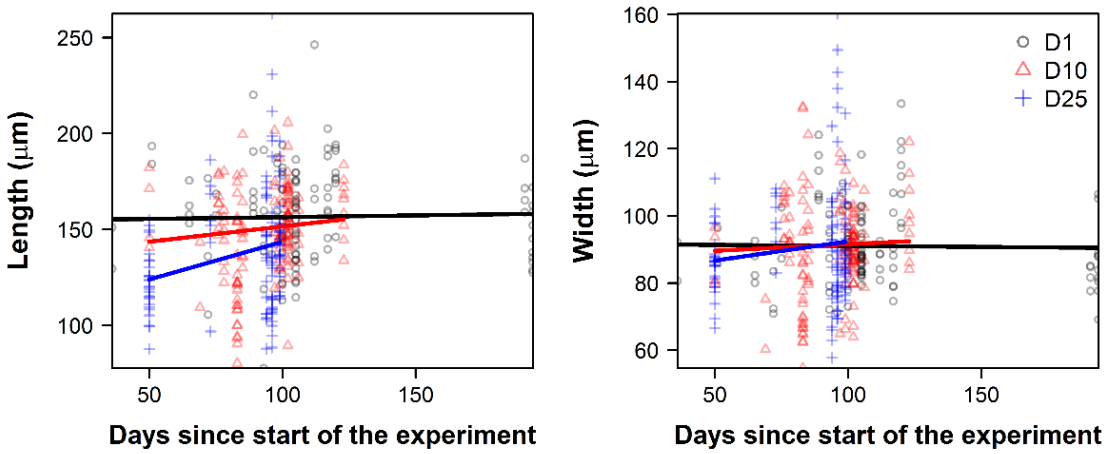

Figure 5.5 The length (left panel) and the width (right panel) of the larvae released in three density regimes during the experiment. The different markers represent data points for the three different densities; the lines show fitted a model. The length of the lines reflects the range of the observed days since the beginning of the experiment for each density.

6 Effects of temperature and salinity on reproduction and growth of hydroid colonies in hydrozoan *Eleutheria dichotoma* - Results

6.1 Effects of salinity and temperature on growth of the hydroid colonies: length of the hydrorhiza (system of stolons)

I observed the huge variation in the patterns elongation of the system of stolons (hydrorhiza) among colonies within the same treatment (**Fig. 6.1, Table A2**), therefore it was hard to detect any differences between treatments. The most homogenous hydrorhiza lengths among colonies were observed in the highest salinity (45) (**Fig. 6.1cfi**). The system of stolons seemed to reach the highest lengths in combination of low temperature (18°C) and medium (35) or low (25) salinity (**Fig. 6.1ab**), but also in combination of high temperature (28°C) and medium salinity (**Fig. 6.1h**). The fastest initial growth of the hydrorhiza seemed to occur in the medium temperature (23°C) (**Fig. 6.1def**), which was clearly visible in the highest salinity (45) (**Fig. A7.1ci,** close to significance when compared with the high temperature of 28°C, **Fig. A.1i**) and medium salinity (35) (when compared to the high temperature of 28°C, **Fig. A.1h**). Conversely, in low temperature the initial growth was much faster in low and medium salinity then in the highest one (**Fig. A.2bc**). In the environmental conditions supporting faster initial growth of the stolon (**Fig. 6.1abdef**), a plateau seemed to be observed at later ages. In other conditions the system of stolons seemed to continuously elongate until the end of experiment.

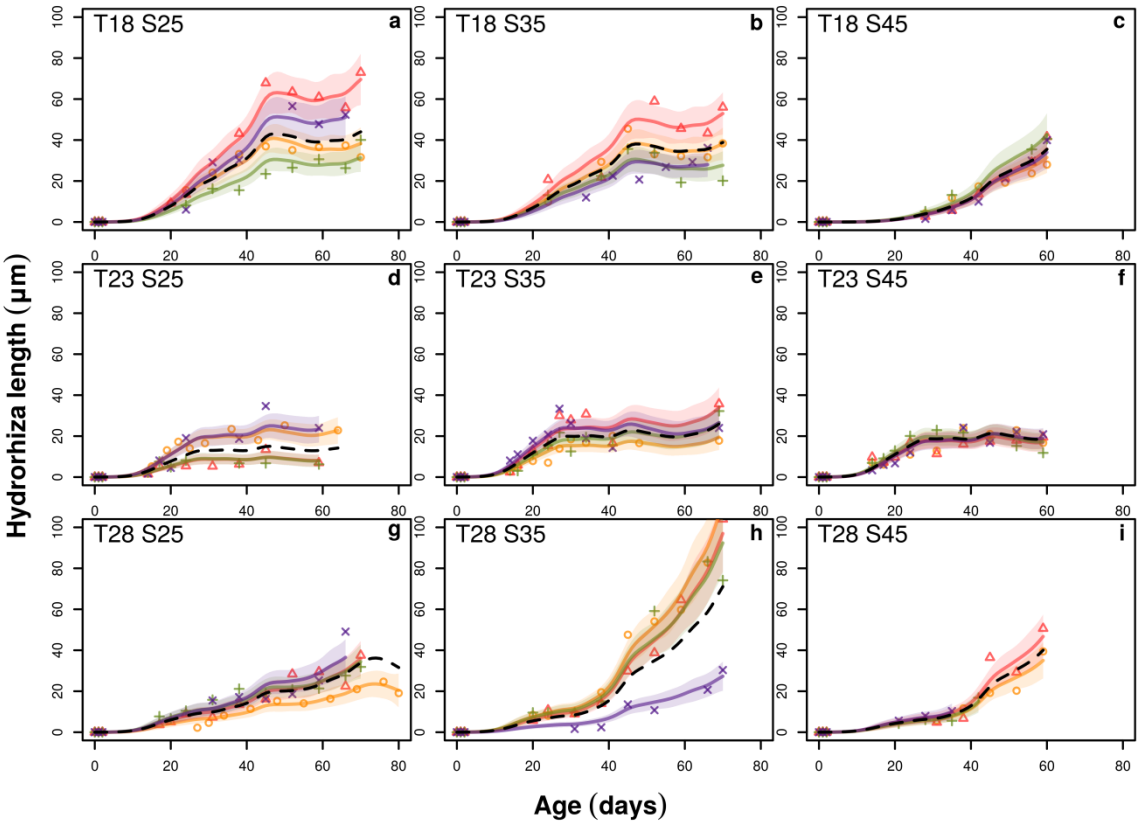

*Figure 6.1 The length of the hydrorhiza (system of stolons) in hydroid colonies of Eleutheria dichotoma at different combinations of salinities (25, 35, and 45) and temperatures (18, 23, and 28°C). Different colors in particular panels represent replicates of hydroid colonies of specific treatment. One colony is prescribed to only one combination of salinity and temperature. The coefficients and significance of the fitted model as well as multiple comparisons between different treatments can be found in supplementary materials (**Tab. A2**, **Fig. A8.1**, and **Fig. A8.2**).*

6.2 Effects of salinity and temperature on growth of the colonies: number of branches of the stolon (hydrocladia)

In almost all experimental treatments I observed similar pattern of hydrocladia (stolonal branches) production: the number of hydrocladia first increased, reached a maximum (sometimes several maxima were present) and then started to decrease before the age of 60 days (**Fig. 6.2**). Both the temperature and the salinity had significant effect on formation of the stolonal branches (**Fig. 6.2, Table A3**). In combination of medium temperature and medium salinity the early increase of number of hydrocladia occurred earlier and/or was higher than in combinations of most temperatures and salinities (**Fig. 6.2e** vs. **Fig. 6.2bdfg, see also Fig.**

A8.1bh, and **Fig. A8.2df**). However, the increase was very similar to the treatment of the lowest temperature and salinity (**Fig. 6.2e** vs. **Fig. 6.2a**).

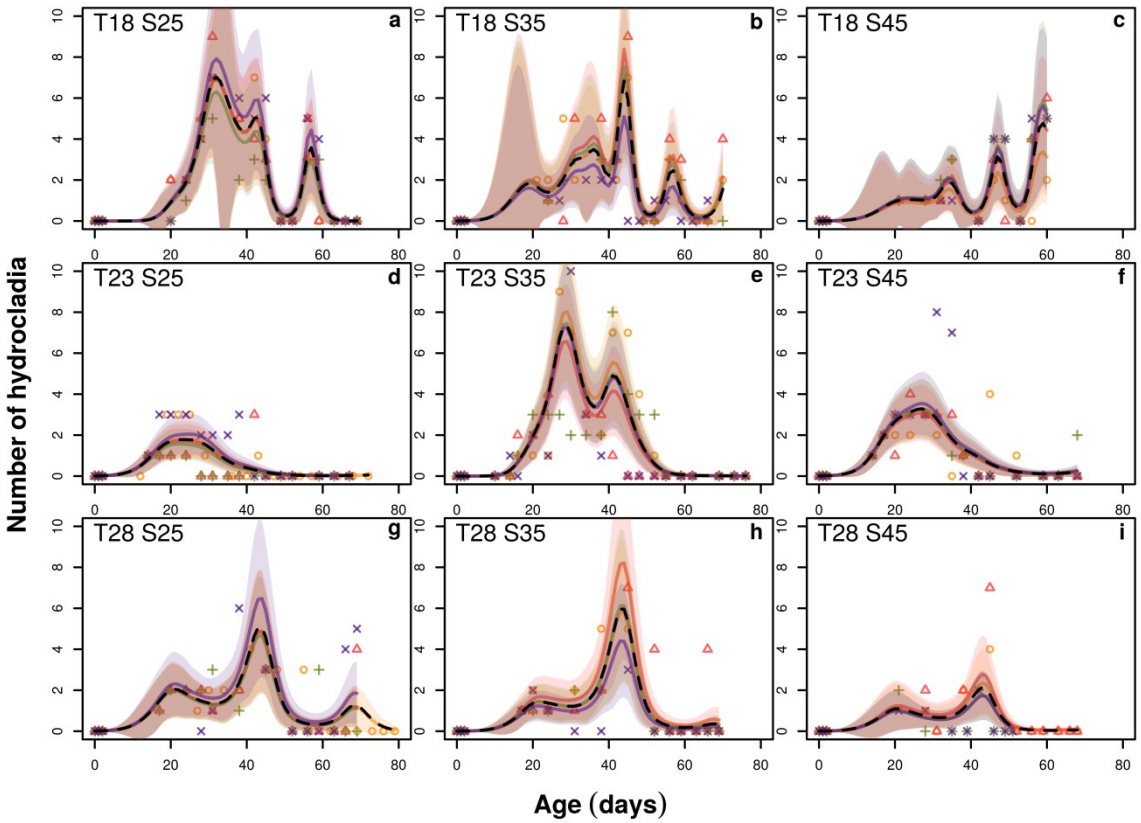

*Figure 6.2 Production of the stolonal branches (hydrocladia) during initial growth phase of the hydroid colonies of Eleutheria dichotoma. The hydroid colonies were reared at the combination of salinities (25, 35, and 45) and temperatures (18C, 23C, and 28°C). Different colors in particular panels represent replicates of colonies of specific treatment. One colony is prescribed to only one combination of salinity and temperature. The coefficients and significance of the fitted model as well as multiple comparisons between different treatments can be found in supplementary materials (**Tab. A3**, **Fig. A9.1**, and **Fig. A9.2**).*

6.3 Effects of salinity and temperature on number of polyps (hydranths) in the hydroid colonies

I observed significant effects of the temperature and salinity on the number of polyps (hydranths) produced in colonies (**Fig. 6.3 Table A4**). In general, the observed changes of polyps' number in different temperature-salinity regimes were qualitatively similar to the results of stolon length (compare **Fig. 6.1** and **Fig. 6.3**). In early age classes of medium salinity treatment the number of polyps increased faster in the medium temperature than in the lowest

(**Fig. A9.1b**) and highest (**Fig. A9.1h**) temperatures (**Fig. 6.3beh**). However, in the treatments with medium temperature, after intensive initial growth, the number of polyps seemed to reach a plateau, whereas the number of polyps in boundary temperatures (18°C and 28°C) seemed to continue to grow or at least to reach a plateau later (**Fig. 6.3**). In the low temperature, colonies reared in low salinity had significantly greater number of hydranths (polyps) than colonies experiencing high salinity (**Fig. A10.2b**), which was observed even in the medium salinity (**Fig. A10.2a**, close to the significance).

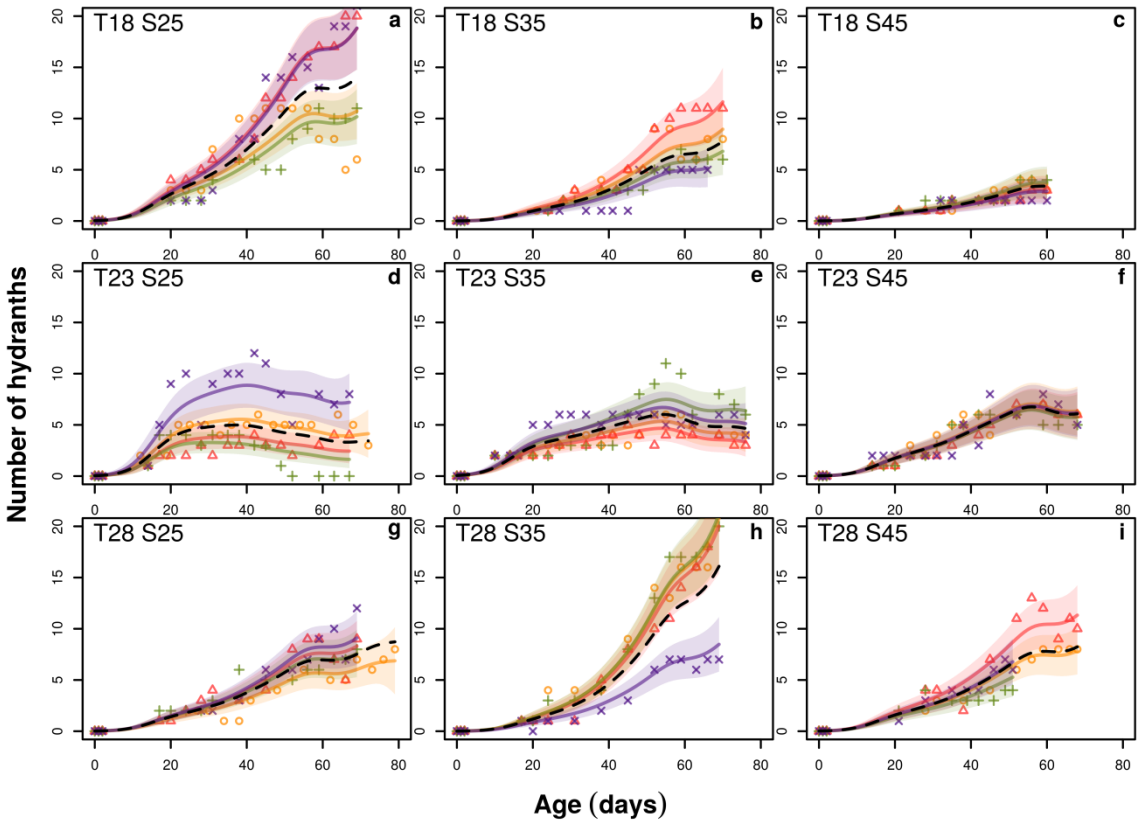

*Figure 6.3 The number of hydranths (polyps) in hydroid colonies of Eleutheria dichotoma at combination of salinities (25, 35, and 45) and temperatures (18C, 23C, and 28°C). Different colors in particular panels represent replicates of colonies of specific treatment. One colony is prescribed to only one combination of salinity and temperature. The coefficients and significance of the fitted model as well as multiple comparisons between different treatments can be found in supplementary materials (**Tab. A4**, **Fig. A9.1**, and **Fig. A10.2**).*

6.4 Effects of salinity and temperature on production of medusae by hydroid colonies

While both, temperature and salinity affect production rates of medusae, the temperature was clearly the strongest factor (**Table A5, Fig. 6.4**). In the average temperature (23°C), the onset of medusae production was the earliest when compared to low (**Fig. A10.1abc**) and high temperatures (**Fig. A10.1ghi**). The high salinity clearly delayed the onset of medusae production in low and high temperatures (**Fig. 6.4ci**), however it was the high temperature that almost completely removed the production of medusae (**Fig. 6.4ghi**). Interestingly, at temperature 23°C, in all salinities, there was a cyclic decrease in the rate of medusae production around 40th and 62nd day (**Fig. 6.4def**) .

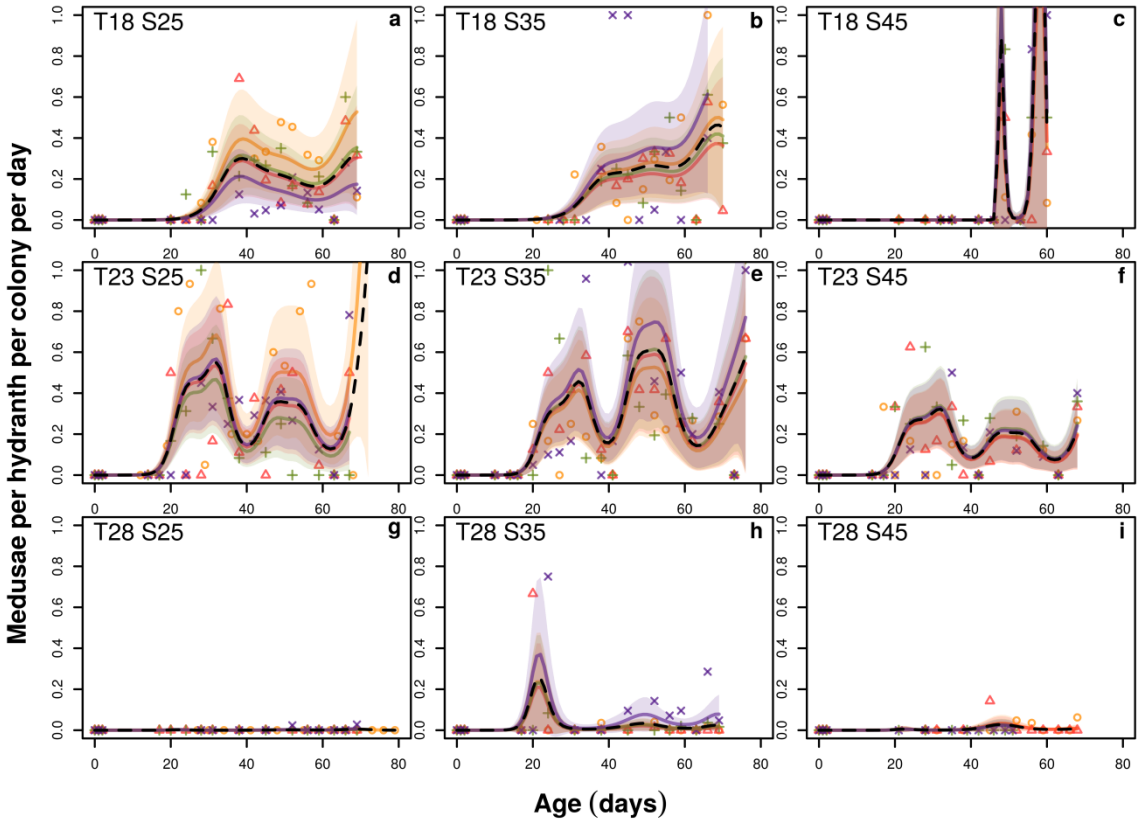

*Figure 6.4 The rate of medusae production by hydroid colonies of Eleutheria dichotoma at combinations of salinities (S: 25, 35, and 45) and temperatures (T: 18C, 23C, and 28°C); Different colors in particular panels represent replicates of colonies of specific treatments. One colony is prescribed to only one combination of salinity and temperature. The coefficients and significance of the fitted model as well as multiple comparisons between different treatments can be found in supplementary materials (**Tab. A5**, **Fig. A10.1**, and **Fig. A10.2**).*

6.5 Effects of temperature and salinity on size of the tentacle knobs

Diameter of the tentacle knobs (see **Fig. A3** in supplementary materials) differed between salinity and temperature regimes, but did not change with age of the colonies (**Table 6.1**, **Fig. 6.5**). In general, the tentacle knobs were the biggest at low temperature (19.9 µm bigger than in medium temperature and 18.3 µm bigger than in high temperature, independently on salinity) and medium salinity (5.9 µm bigger than in low salinity and 7.7 µm bigger than in high salinity; independently on temperature). There were no significant differences in size of tentacle knobs between colonies reared at high and medium temperatures.

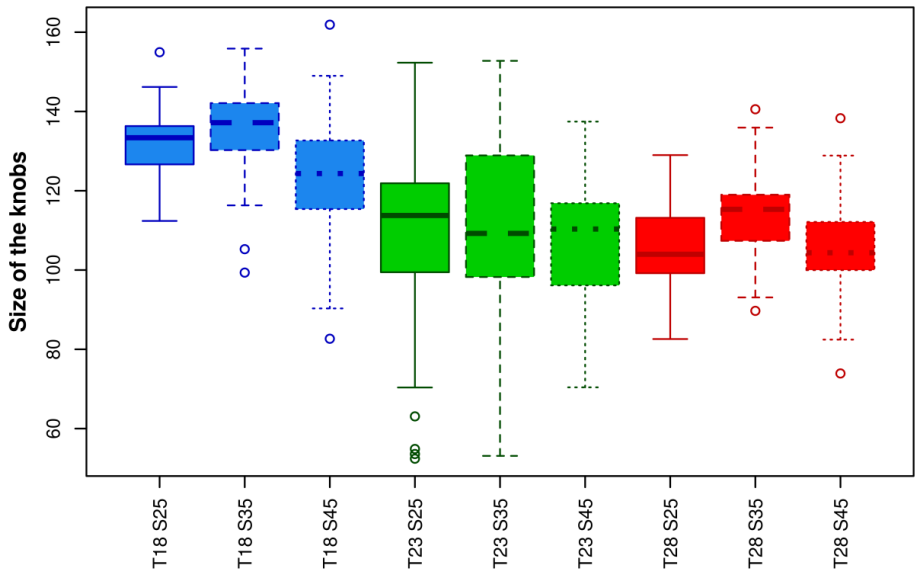

Figure 6.5 Comparison of the size of tentacle knobs (in µm) in individual hydranths (polyps) growing on hydroid colonies of Eleutheria dichotoma at combination of temperature and salinity. Colours represent different temperatures (T: 18, 23, and 28 °C) and lines styles represent different salinities (S: 25, 35, and 45).

Table 6.1 Comparison of the diameter of tentacle knobs in individual hydranths (polyps) growing on hydroid colonies at different combinations of temperature and salinity. Results of linear regression of tentacle knobs diameter [µm]; The treatments with salinity 35 and temperature 23°C were used as reference (control) variables

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	108.60	3.38	32.13	0.0000
Salinity (25)	-5.93	2.73	-2.17	0.0308
Salinity (45)	-7.71	2.86	-2.70	0.0074
Temperature (18°C)	19.90	3.16	6.29	0.0000
Temperature (28°C)	-1.60	3.09	-0.52	0.6046
Age	0.13	0.08	1.61	0.1097

6.6 Effects of salinity and temperature on expression of heat shock proteins (HSP) in hydroid colonies and medusae

Due to insufficient protein concentration, many samples were rejected from further analysis. Finally, I used only samples from hydroid colonies reared in high temperature (28°C) and from medusae reared in all salinities and two temperatures (18°C and 23 °C). Colonies reared at high temperature and medium salinity (35) had significantly lower expression of HSP than colonies reared at low (25) and high (45, close to significance) salinity (**Table 6.2A**). The effect of salinity on expression of HSP70 was not visible in medusae, however medusae maintained in low temperature (18°C) had significantly lower expression of HSP70 than in the control group reared in 23°C (**Table 6.2B**).

Table 6.2 Coefficient and significance of model fitted to HSPs expression A) in hydroid colonies reared in high temperature (28°C); B) in medusae reared in temperatures 18°C and 23°C (reference). In both cases, three salinities were considered: 25, 35 (reference) and 45.

A)	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	15.51	5.07	3.1	0.0281
Salinity (25)	21.52	6.54	3.3	0.0218
Salinity (45)	14.15	6.54	2.2	0.0829
B)	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	72.20	2.31	31.2	<2e-16
Salinity (25)	0.57	3.13	0.2	0.8558
Salinity (45)	-1.84	3.85	-0.5	0.6353
Temperature (18°C)	-6.42	2.75	-2.3	0.0226

7 Discussion

7.1 Effects of environmental stress (ES) on medusae in hydrozoan *Eleutheria dichotoma*

7.1.1 Effects of ES experienced by hydroids on life histories of medusae

I found that the salinity experienced by hydroid colonies may affect the onset of reproduction and the reproductive rates of medusae. The medusae that had detached from hydroid colonies in low salinity and were transferred to medium salinity matured sexually later and had lower larval production rates (see Chapter 3). Conversely, the asexual reproduction of these medusae increased. My other results (Chapter 4) show that temperature experienced by hydroid colonies significantly affects initial size of primary medusae. Experience of high temperature during initial development as a bud attached to hydranth decreased initial size of medusae but did not affect the age at first asexual reproduction. Furthermore, survival of these medusae increased, which can be explained by, so called, hormesis effect; often observed in other invertebrates e.g. *Drosophila sp.* (Hercus et al. 2003). Conversely, size of medusae that experienced low temperature during initial development increased almost two times. The low temperature delayed both asexual and sexual maturity of medusae. The observed effects support temperature-size rule, stating that ectotherms develop longer and tain greater size when reared at low temperatures (Atkinson 1994).

Medusae that detached from hydroid colony at low temperature and were transferred to 23°C matured sexually earlier than controls (medusae reared at 23°C). These results suggest that medusae were not only bigger but also more advanced in development. Such progress in development might be obtained by prolonged period of connection of medusa bud to hydranth. This connection might enable transfer of resources from hydroid colony to a bud and continuous growth of a bud. The longer period when medusa buds stay attached to a hydranth might increase survival of medusae during adverse conditions (low temperatures during winter, low

food level). Conversely, in the spring they might be a source of well-developed medusae that can quickly initiate reproduction.

The obtained results support hypothesis nr. 1 according to which environment experienced by hydroid colony may affect initial size and maturity of primary medusae. Both the change of salinity as well as the change of the temperature between hydroid and medusa life forms may affect life history of medusae. It can thus be hypothesized that the connection of the medusa buds with the hydranths might have a protective effect on the buds by providing additional nutrients or even stem cells (Wittlieb et al. 2006) in adverse conditions. The mechanism linking the effects of the environment experienced by hydroid colonies to the life history patterns of the medusae could be analogous to the parental effects observed in other species, whereby the environment experienced by a parent (mother) improves the fitness-related traits of the offspring (LaMontagne and McCauley 2001). To my knowledge this is the first study showing such effect in hydrozoa.

7.1.2 Effects of environmental stress (ES) on the modes and the rates of reproduction in medusae

The results of Experiment I, show that salinity affects reproductive modes and rates of medusae. The medusae of *E. dichotoma* reared in low salinity (25) initiated budding earlier, which suggests that asexual reproduction should dominate in these conditions. Importantly, the increased investments in asexual reproduction resulted in delayed sexual maturity and lower larvae production rates. The increased budding of medusae in low salinity has also been observed in some estuarine species (Ma and Purcell 2005; Holst and Jarms 2010; Canepa et al. 2014) but other species decrease asexual reproduction at these conditions (Rippingale and Kelly 1995; Purcell et al. 1999). Generally, coping with hypoosmotic conditions involves less energy as it occurs by simply lowering internal ionic concentration (Amado et al. 2011; Rivera-Ingraham and Lignot 2017). The saved energy can be invested elsewhere, for example in

reproduction. Asexual reproduction allows rapid propagation as it is a fast developmental process that appears to be dependent on the proliferative potential of somatic cells, and can be related to regeneration (Tanaka and Reddien 2011; Dańko et al. 2015). The production of multiple clonal individuals might enable the rapid colonization of new environments that arise through salinity change (Schlesinger et al. 2010; Canepa et al. 2014).

I observed that sexual reproduction is more likely to occur in medium salinity (35) rather than in low salinity (25), and do not occur at high salinity (45). The medusae reared in medium salinity reached sexual maturity the earliest and produced larvae at the highest rate. These findings suggest that a salinity of 35, which is standard sea water and is typically experienced by *E. dichotoma* in the wild (Borghini et al. 2014), may be close to optimal for sexual reproduction and favourable for larval development and survival.

High salinity clearly had negative effects on the maturation and reproductive rates of *E. dichotoma*: in high salinity conditions, asexual maturation was delayed, the budding rate was lowered, and planula production never occurred. The reduced rates of reproduction in these conditions might be at least partially a consequence of the lower allocation of energy to reproduction due to the increased costs of coping with osmotic stress (Lubzens et al. 1985; Folino-Rorem and Renken 2018). In some cnidarian species high salinity decreases asexual reproduction (Purcell et al. 1999), but the effects are often combined with temperature (Purcell 2007).

The results of the Experiment II (Sub-Experiment 1 and Sub-Experiment 2) show that both low as high temperature decrease the rates of reproduction. Both medusae budding rates and larvae production rates were the highest at temperature 23°C and decreased with contribution of low temperature during medusa life. Even though medusae reared at temperature 12°C reached greater size at maturity than medusae reared at 23°C, they produced

buds with a lower rate. This may suggest that buds developed slowly or they were longer attached to medusae. Similarly, the experience of low temperature during initial development of medusa bud decreased budding rates. The common for ectotherms relation that bigger organisms have higher fecundity (Honěk 1993) was not confirmed for *Eleutheria dichotoma*. Larvae production rate at temperature 12°C was very low suggesting that such conditions are inappropriate for sexual reproduction.

The results of Experiment III show that population density also affects reproductive rates and modes of medusae. The sexual reproduction rate was the highest in the moderate density conditions (D10). The increase of investments in sexual reproduction seemed to occur at the cost of asexual reproduction as there was a significant decrease in the budding rate with density. However, further investigations are needed to test if such a trade-off really exists, especially that the negative effects of overcrowding at the highest population density are likely to overlay the positive effects on sexual reproduction.

At all density regimes there is a peak of the sexual and vegetative reproduction rate at early life stages. The medusae that were maintained individually reached their highest age-specific asexual reproduction rate at the beginning of life, and their highest larvae production rate shortly thereafter. My results suggest that under higher density conditions (D10 and D25), medusae may reach their peak of sexual reproduction rate slightly earlier, and could reach sexual maturity earlier as well. Even though, both medium and high population densities have no detectable effects on medusae size at maturity, they might have affected size of their offspring (planula larvae). The production of smaller larvae in D10 and D25 might be associated with earlier sexual maturity among the medusae (Fig. 5.4, right panel). It is likely that the medusae maintained a high individual growth rate at the expense of offspring size. However, the sizes of the larvae increased with the age of the medusae (Fig. 5.5). This increase is likely a byproduct of the experimental design, as the population densities in the research vessels (and

their effects on life histories) decreased with time. The production of bigger larvae may be adaptive, because bigger larvae could have more energy reserves that allow them to spend more time swimming before settling. Thus, bigger larvae may be more likely than smaller larvae to find a habitat suitable for a wider dispersion (Marshall and Bolton 2007).

The results of the four experiments support hypothesis nr. 2 according to which environmental stress (here: salinity, temperature and population density) may change reproductive modes and rates in medusae. I showed that planulae production increases with deterioration of environmental conditions, e.g. increased population density. The production of motile life form in unfavorable conditions allows for dispersal and searching for new environments. Conversely, the highest medusae production rate occurred at low population density, low salinity and low/medium temperature. Production of medusae buds in these conditions allows for rapid colonization of new environment.

7.1.3 Effects of environmental stress (ES) on consecutive generations of medusae

The results of Experiment I show that salinity levels may affect the patterns of investment in reproductive modes in consecutive generations of medusae. Although the release of the first bud was delayed and the rates of bud production decreased with each generation, the medusae became sexually mature earlier and (in low salinity) they produced more larvae with each generation. Theoretically, the observed gradual shift from asexual to sexual reproduction with each generation could be explained as a strategy for avoiding damage accumulation. Asexual reproduction seems to be more efficient in the short run; however, it may lead to the accumulation of deleterious mutations in a process known as Muller's ratchet (Felsenstein 1974), and thus to a gradual deterioration of the genetic line (Loewe and Cutter 2008). The most obvious way to avoid such deterioration is to allow for recombination through sexual reproduction and subsequent selection (Maynard Smith et al. 1988). However, this mechanism cannot be effective in *E. dichotoma,* which is a hermaphrodite that self-fertilizes. Reproductive

organs in *E. dichotoma* are placed inside the brood chamber and fertilization occurs internally, which excludes the sense of existence of males (Hauenschild 1956).Conversely, the results of Experiment II (Sub-Experiment 1) show that the larvae production rate tends to decrease, while budding rate do not change significantly with generation at temperature 23°C.

The results of my study support hypothesis 3 according to which ES may change reproductive modes and rates with generation of medusae. The gradual change of reproductive mode with generation might be a result of adaptation to seasonal changes, or to internal rhythms on genetic basis. Hydrozoans are known to have circannual internal rhythms, which affect the reproductive phases of their hydroid colonies (Bavestrello et al. 2006). It is theoretically possible that such internal rhythms are present in medusae and influence their reproductive strategies over the course of a season.

7.1.4 Environmental stress (ES) may promote senescence in medusae

The results of Experiment I show that, under laboratory conditions, salinity may affect the survival of medusae. Salinity levels that were lower or higher than the assumed optimum of 35 were associated with lower survival. These finding suggests that there may be a trade-off between resource allocation to osmotic adaptation and general maintenance resulting in faster ageing, which supports hypothesis nr. 4. The potential mechanism may be associated with increased metabolic rate at marginal salinities and resulting oxidative stress from free radicals (Rivera-Ingraham et al. 2016). My findings further indicate that the rapid decline in survival in low salinity seems to begin just after the peak of asexual reproduction. These results suggest that the medusae gradually shift their resources from investments in maintenance and reproduction to expenses connected with osmotic stress.

Low temperature prolonged medusae life suggesting that *E. dichotoma* may potentially survive low winter temperatures, even though it has never been observed during winter (Hadrys

et al. 1990). Indeed, other studies show that in laboratory conditions, hydrozoan medusae survive low temperatures and live more than one year. Also field studies show that some hydrozoan medusae can overwinter (Purcell 2017). In opposite to this, the results of Experiment II (Sub-Experiment 2) show that medusae reared at high temperature had low reproductive rates and low survival, which may lead to disappearing of this life form during summer.

Experiment III showed that high population density have clear effects on the survival patterns of medusae in laboratory conditions. As the density level increased, the medusae lived shorter lives, and had an increased rate of aging, which supports hypothesis nr. 4. There are two possible explanations for these patterns, which are not mutually exclusive. The faster rate of aging could be (1) an outcome of direct negative effects of density on life history traits, such as via the accumulation of toxic metabolites in wells; or it (2) could be an indirect effect of trade-offs between investments in sexual reproduction and in tissue maintenance.

Both explanations imply that there is a link between population density and individual deterioration, and hence earlier senescence. In my experiment, at every density level, the rise in mortality was accompanied by physiological deterioration. However, the patterns of deterioration depended on the degree of density. After reaching its highest sexual reproduction rate, the medusae maintained in high population densities (D25) had an empty and expanded umbrella with short tentacles (personal observation). This outcome could reflect the direct costs of the increased rate of sexual reproduction, including overcrowding; which could in turn be linked to an increased rate of aging. Interestingly, medusae reared under medium density conditions (D10) did not show a pattern of deterioration, which suggests that this level of density is more tolerable for the species. Like the medusae maintained individually (D1), medusae D10 shrank with age, and eventually dissolved.

The mortality rates decelerated at later ages, which may have been an outcome of (1) decreasing population density with time, or/and (2) hidden heterogeneity in levels of individual

frailty (Vaupel et al. 1979; Carey et al. 1995). The individuals of a single population are likely to differ in their susceptibility to all causes of death, even if the population is genetically homogenous. On the one hand, even tiny differences between individuals can lead to a substantial degree of heterogeneity in stressful conditions, but on the other, stressful conditions can cause less robust individuals to be eliminated from the population more quickly. The fitted model showed that the highest degree of heterogeneity in unobserved mortality risks was present in the lowest density conditions. This finding can be explained by the high degree of individual variation in resource allocation strategies, and is supported by the higher degree of variation in other traits (i.e., asexual and sexual reproduction rates, **Fig. 5.2**).

The decrease in the density level within each of the experimental treatment did not lead to a convergence of mortality rates across the different experiments. I observed that the mortality plateau was highest in the highest density conditions, and was lowest in the lowest density conditions. There are two potential explanations for this effect: (1) that differences in population's hidden heterogeneity greatly shape mortality patterns later in life; and (2) that the harmful effects experienced early in life in high-density conditions cannot be overcome later in life, when density substantially decreases. This second explanation is clearly supported by the increase in the rate-of-aging parameter with population density (see patterns of survivorship and mortality, **Fig. 5.3**).

7.2 Effects of environmental stress (ES) on hydroid colonies in hydrozoan *Eleutheria dichotoma*

7.2.1 Effects of environmental stress on growth of hydroid colonies

The results of Experiment IV suggest that temperature and salinity affect growth and reproduction of hydroid colonies. The most intensive growth occurs in young hydroid colonies, which was expressed not only directly by the rate of stolonal elongation (**Fig. 6.1**) and

increasing number of hydranths (**Fig. 6.3**), but also by production of stolonal branches (hydrocladia) (**Fig. 6.2**). The number of hydrocladia initially increased in all colonies and after reaching a maximum at some (still relatively early) point of colonial life, it started to decrease. In low temperature the process of the colony growth seemed to last longer than in other regimes (**Fig. 6.2abc**), because the production of stolonal branches had not stopped during the experiment. These observations correspond to the lack of plateau in stolonal length and number of hydranths observed for these regimes.

In my experiment, colonies maintained at medium temperature seemed to reach plateau of stolon length (**Fig. 6.1def**) corresponding to observed morphological changes of the older stolon (personal observation). It was previously shown that high temperature in combination with good nutritive state of hydroid colony have the greatest positive influence on a stolonal growth (Wyttenbach 1968). The efficiency of food ingestion is likely to decrease with increasing temperature (Schroeder and Callaghan 1982) which may result in prolonged production of at low temperatures (**Fig. 6.2**).

Hydroid colonies of *E. dichotoma* did not experience dramatic deterioration in growth despite experiencing extreme temperatures and salinities. The lack of deterioration suggests that an average hydroid colony has much broader spectrum of tolerance to environmental conditions. However, it must be noted, that there was a remarkable variation in patterns of growth in different colonies (**Fig. 6.1** and **6.3**), which seemed to decrease with increasing salinity. Although each experimental colony was risen from different larvae, the effects of genetic variance on growth patterns, remarkable in other species (Wyttenbach 1968), should not be the case in this experiment. *E. dichotoma* is self-fertilizing hermaphrodite and all planula larvae that initiated the hydroid colonies were clonal (originated from the same clone). Possibly, the observed variation between hydroid colonies can be related to the post-fertilization period, i.e. a stage of early development of embryos in a brood pouch on the aboral side of medusae.

For example, my another study (Chapter 5, **Fig. 5.5**, see also (Dańko et al. 2018a) shows that the size of larvae at birth (day of release from medusa) may be influenced by environmental factors experienced by medusae during development of embryos, which can be seen as a kind of maternal effect. In many cnidarians, the size of larvae determines their survival and success of metamorphosis into polyp stage (Marshall and Bolton 2007) and might potentially affect performance of the hydroid colonies.

I showed that stolon length correlates with the number of hydranths (compare **Fig. 6.1** with **Fig. 6.3**) and the most harmful conditions are related to the highest salinity which is similar to previous studies on another colonial hydrozoan (*Clava multicornis;* Kinne and Paffenhöfer 1966). Despite these similarities, results of Kinne and Paffenhöfer (1966) are qualitatively different from mines, indicating different ecological optima of both species. They observed that the highest length of the stolon and number of hydranths (both measured after 39 days) occur in medium salinities at temperature close to the species optimum (17°C). In my study, the highest growth of colonies and the highest numbers of hydranths were observed in the regimes of low-medium salinity and low temperature (**Fig. 6.1ab, Fig. 6.3ab**) as well as medium salinity and high temperature (**Fig. 6.1h, Fig. 6.3h**).

The combination of low temperature and low or medium salinity not only increased the number of hydranths and stolon length, but also size of hydranths via enlargement of tentacle knobs (**Fig. 6.5**). These changes are consistent with temperature-size rule, according to which ectotherms may grow slower with declining temperature, but reach larger size due to postponed maturity (Atkinson 1994; Weetman and Atkinson 2004; Kozlowski et al. 2004), which was also observed in my study (**Fig. 6.3abc** vs. **Fig. 6.3def**). The similar delay of maturity was observed in other organisms including hydrozoan species, e.g. in hydrozoan with solitary polyp life form, *Moerisia lyonsi* the temperature affected time of development of both polyps and medusae buds (Ma and Purcell 2005). Furthermore, my study (Chapter 4, **Fig. 4.3**) shows that medusae buds

produced by hydroid colonies at low temperature (12°C) are bigger at the day of release, which can result from their longer development in the form of bud attached to hydranth.

My study supports hypothesis nr. 5 according to which ES should affect growth of hydroids. I showed that temperature is the most important factor affecting growth and the greatest colonial growth was observed in combination of low temperature and low/medium salinity. These results suggest that natural hydroid colonies might thrive during mild winter temperatures.

7.2.2 Production of medusae by hydroid colonies

The production rate of medusae buds was the highest in temperature 23°C. In 28°C, the primary medusae were hardly produced, and in 18°C their production seemed to be in general not only lower (non-significant observation), but also significantly later was its onset. In contrast to this, stolon growth was higher at low temperature, rather than in medium temperature. The results support hypothesis nr. 4, according to which there may be a trade-off between production of medusae and growth of the colony. The trade-off can be explained in the light of optimal resource allocation theory (Kozłowski 1992, 2006; Stearns 1992). According to the theory, the resource allocation strategies and thus observed life histories depend on such factors as amount of available resources or extrinsic mortality. Higher investment in the growth of the hydroid colony can be interpreted as an investment in survival of the clone (genet) in unfavorable environmental conditions, because polyp life form seems to be more resistant than medusae (Chapter 6, see also (Dańko et al. 2015). Similar results, but for different temperatures, were observed in solitary polyps of Scyphozoa, where higher temperatures enhance budding of scyphistomae and decrease their strobilation rate (Willcox et al. 2007; Sokołowski et al. 2016).

The main role of producing (primary) medusae by hydroid colonies is to enable sexual reproduction, which is only possible in medusa life form. My studies show that the highest rate of sexual reproduction in medusae occurs at medium temperature (23°C) and medium salinity

(35) (Chapter 3, **Fig. 3.2**), whereas high temperature (28°C) and high salinity (45) impair sexual maturation and reproduction of medusae (Chapter 4, **Fig. 4.1**; Chapter 3, **Fig. 3.1**). In my experiment, high temperature markedly reduced the rate of medusa buds production as most of the hydranths did not develop buds (personal observation, see also **Fig. A7**, there are no medusa buds attached to hydranths). Morphologically, they resembled hydranths intensively growing on the periphery of hydroid colony, which usually do not reproduce (see also Hall and Hughes 1996). In contrast to this, growth of the colony and number of hydranths were not strongly affected (**Fig. 6.1** and **6.3**). The decreased medusae buds production at high temperature suggests that hydroid colonies relocate resources from budding of medusae (reproduction) to maintenance and growth of the colony (vegetative growth and production of hydranths). Similarly, the studies on *Clava multicornis* show decreased production of medusae in high/low temperatures and salinities (Kinne and Paffenhöfer 1966).

Interestingly, at medium temperature (23°C) there were two significant drops in medusae production rate around 40^{th} and 62^{nd} day, visible in all salinities. It must be noted that these fluctuations do not correlate with feeding regimes (feeding two times per week, colonies in different salinity regimes started in different calendar days). They may reflect periods of development of medusae buds attached to hydranths, suggesting that their development might be synchronized between hydranths within colony.

The results of my study support hypothesis nr. 6 according to which ES affects production of medusae by hydroid colonies. Production of medusae may be compromised by growth of the colony and production of hydranths. The highest production of medusae occurred at medium temperature and medium salinity, which are conditions optimal for sexual reproduction. Furthermore peaks of medusae buds production occurred in regular periods, suggesting that it can be synchronized within a colony. The increased production of hydranths was observed at high temperature.

7.2.3 Expression of heat shock proteins (HSPs) in hydroid colonies and medusae

The measurement of expression of HSPs in hydroid colonies and medusae shows no clear results. Many samples were discarded during analysis due to not sufficient protein content. Expression of HSPs in hydroid colonies and medusae measured for the remaining samples suggests that increased production (expression) of HSPs in response to changes in salinity occurs solely in hydroid colonies (**Table 6.2A**). This could be one of the reasons of their higher resistance to extreme conditions compared to medusae. The previous studies on another marine invertebrate (sea cucumber) show that these organisms have adaptive molecular response to change of ambient salinity (Dong et al. 2008). Unfortunately, I was able to obtain results only for the hydroid colonies maintained in the highest temperature. However, results obtained for medusae (only from medium and low temperature) (**Table 6.2B**) suggest that the temperature may be also a strong factor enhancing production of HSPs in *E. dichotoma*. Certainly further research is needed.

I could not definitely support hypothesis nr 7. according to which medusae and hydroids might differ in expression of HSPs and consequently have higher resistance to environmental conditions. The higher resistance of hydroid colonies compared to medusae may be explained by the presence of mechanical protection through covering perisarc.

8 Conclusions

My results showed that *Eleutheria dichotoma* seems to belong to a group of hydroids that are capable of changing their reproductive activity over the year. The pattern of growth, survival, and reproduction depended on the salinity, food levels, and population density, but the impact of temperature seemed to be the strongest, especially for hydroid colony. I showed that *Eleutheria dichotoma* might thrive during cold or mild season because low and medium temperature as well as low and medium salinities speed-up elongation of the stolon and increase production of both hydranths and medusae. Conversely, the animal might regress during summer, as high temperatures and high salinities lead to decreased production of medusae buds, decreased budding of secondary medusae and lower survival of medusae.

Response to low temperatures, resembling winter conditions

Hydroid colonies reared at temperature 18°C delayed onset of medusa buds production, but the rate of medusae buds production was at similar level as in medium temperature (23°C). Furthermore the colonies, as well as particular hydranths grew to bigger sizes at low temperature, which is in accordance with the temperature-size rule (Atkinson 1994). Conversely, my results also show that medusae that detached from hydroid colonies reared at temperature 12°C were bigger at the day of release and matured earlier when transferred to temperature 23°C. Surprisingly their reproduction rates did not differ from controls reared continuously in medium temperature.

Response to mild temperatures resembling spring conditions

The highest production of medusae by hydroid colonies occurred in temperature 23°C, which corresponds to spring and mild summer in north Mediterranean. I showed that increased production of medusae might be a response to the yearly patterns of phytoplankton and zooplankton maxima, which are result of changing physical conditions (increasing

temperatures, longer light period) (Sommer et al. 2012). Furthermore, production of sexually reproducing medusae would be beneficial for hydroids as it leads to emergence of planula larvae. The planula can actively search for a site to settle and start new colonies possible in better environmental conditions.

Indeed, medium temperature (23°C) in interaction with salinity allow medusae for the highest rates of reproduction. *Eleutheria dichotoma* is the only known species of budding hydromedusae that simultaneously reproduces both sexually and asexually. Following previous study (Schierwater and Hadrys 1998), I showed that the modes and the rates of reproduction can be modified by environmental conditions like salinity, temperature and population density. My study showed that in medium salinity (35), the medusa budding rates were similar to larvae production rates, however in low salinity (25), medusa budding rates increased and larvae production rates decreased. Low salinity conditions might occur in small water reservoirs like tidepools after the rainfalls. My study shows that *Eleutheria dichotoma* may take advantage from low salinity conditions by increasing medusa budding rates (asexual reproduction), which may lead to high population density. I found also significant effect of salinity experienced by hydroids on reproductive rates of medusae. Experience of low salinity levels by hydroids decreased larvae production rates of primary medusae. These findings, suggest a possibility of a transfer of environmental information from hydroid colonies to medusa buds.

My results show that medusae may have flexible life history responses to different population densities. Thus, the reproductive/propagation strategies of medusae may occupy different places along the r/K-strategies continuum. In laboratory conditions characterized by low population densities, the animal invests in the strategy of clonal propagation (the medusa stage most closely fits the definition of the r-strategy). However, it attempts to escape unfavorable conditions (e.g. high population densities) by producing offspring (larvae) that are able to disperse and settle a new hydroid colony (the polyp stage most closely fits the definition

of the K-strategy). The production of a large number of short-lived planula larvae increases the survival chances of a genet in the polyp stage.

All density regimes were characterised by a peak of the sexual and vegetative reproduction rate occurring at early life stages. The medusae that were maintained individually reached their highest age-specific asexual reproduction rate at the beginning of life, and their highest larvae production rate shortly thereafter. My results suggest that under higher density conditions (D10 and D25), medusae may reach their peak sexual reproduction rate slightly earlier, and could reach sexual maturity earlier as well. The very early peaks in the life course of both sexual and asexual reproduction may be attributable to a process of adaptation to the large amount of food available at the beginning of the vegetation season. The field studies from temperate regions have shown that the highest levels of diversity and density of different species of hydromedusae are reached in the late winter/early spring period, which coincides with the yearly maxima of phytoplankton and zooplankton (Costello and Mathieu 1995). *Eleutheria dichotoma*, with its rapid reproductive response to medium temperature, might contribute to this diversity of hydrozoan species early in the spring.

Response to high temperature, simulating summer temperature regimes

In high temperature (28°C, independently of experienced salinity) and high salinity (45, independently of experienced temperature) hydroid colonies continued to grow and produce new hydranths, while medusa bud production was negligible. This suggests the shift of investments from budding medusae, which could not reproduce sexually in high temperature to more resistant to environmental conditions hydroid colony. Analysis of heat shock proteins expression suggested that hydroid colonies might be more resistant to environmental stress than medusae. Consequently, in unfavorable conditions they are presumably the main source of medusae in the population.

The medusae reared at temperature 28°C had low reproductive rates and short lifespans. Consequently, medusae might disappear during summers with high temperatures, leading to refinement of the population to hydroid colonies. The spring peak of zooplankton leads to decline in phytoplankton biomass resulting in mid-season biomass minimum (clear water phase) (Sommer et al. 2012). In small water reservoirs like tidepools these mechanisms may be even more pronounced as they are highly dependent on mechanisms regulating population density. The seasonally occurring medusae are susceptible to variable environmental conditions, which may shorten their lifespan. The higher investments in planulae production at medium and high population density come at a cost of lower asexual budding rates and of lower investments in tissue maintenance, which in turn result in increased mortality and aging rates.

Response to deterioration of conditions

I showed that high temperature experienced by hydroid colonies increased larvae production rate of primary medusae. Sexual reproduction results in production of larvae which are motile and can seek for a better environment to start polyp generation. Passing to polyp phase was observed also within generations of medusae. My results on medusae reared at medium and low salinity for three generations showed that the medusae may have increased their investments in sexual reproduction and decreased their investments in asexual reproduction. The increased investments in the production of planula larvae indicated that the medusae were transitioning to a benthic polyp phase. I showed that planula larvae produced in highest population densities were bigger than larvae produced in low population densities. This suggests that they might gain additional resources during their development as embryos in a brood pouch. Because the larvae are lecithotrophic (incapable of feeding themselves) (Collins 2002; Marshall and Bolton 2007), brooding embryos in a brood pouch is a critical means of supplementing resources. The byproduct of this strategy is that larvae get additional chances for dispersal, because medusae carrying embryos may float passively to different environments.

Heterogeneity of hydroids and medusae

In the Experiment IV I showed that there is a remarkable variation in growth patterns among hydroid colonies experiencing the same environmental conditions. The presence of substantial variation in life history traits reflects high plasticity and stochasticity of colonial development, which cannot be simply explained by genetic variance, because *E. dichotoma* is self-fertilizing hermaphrodite. However, other studies on *E. dichotoma* show that, in contrast to theoretical expectations, there is unusually high level of clonal diversity within and among populations in the wild. Furthermore, the degree of genetic divergence was higher within populations than between them. Possible explanation may be coadapted gene complexes with local genotypes under different selective regimes, as well as high mutation rate leading to mutation divergence (Ender 1997). Another explanation of that huge variation of life history patterns in single clone of *E. dichotoma* may be a byproduct of bet-hedging strategy (Botero et al. 2015). It can be seen as a strategy of minimizing fitness variance across the whole range (likely to experience) of environmental conditions or/and a strategy of taking benefits from alternative environmental scenarios (Starrfelt and Kokko 2012; Botero et al. 2015).

I show that the age-specific rates of asexual reproduction of medusae have unexpected patterns in late life stages (**Fig. 5.3**). While the sexual reproduction rate in all of the density regimes followed a typical senescence pattern and decreased with age, the asexual reproduction rate tended to increase later on (**Fig. 5.3**, left panel), similarly I showed a potential substantial variation in individual mortality rates leading to bending of the mortality curve for the total population. This increased asexual reproductive rate at later ages might be a result of decreasing density in the experimental wells due to deaths (as the dead medusae were not replaced) or the effect of sampling noise (as few individuals were left; notice the widening confidence intervals in **Fig. 5.3**).

The last but not the least, the important source of heterogeneity can be an extraordinary feature of *E. dichotoma* medusae, whereby a developing bud may overgrow the aging medusa mother, and can sometimes even absorb it. Such a behavior can lead to increased life-span and reproduction rates. Following the procedures of my experiment I removed such medusae (marked as censored at time of removal in the data), however, in some rare cases, few individuals could have been overseen.

9 Summary

This PhD Thesis investigates the effects of environmental stress on life history of hydrozoan with complex life cycle, *Eleutheria dichotoma*. The life cycle of this species comprises both benthic, colonial polyps and motile medusae. The subsequent appearance of life forms can be a result of different strategies of resource allocation to maintenance of the colony and its reproduction. For example, the increased investment in growth of the colony might be compromised by decreased investment in production of medusae. *Eleutheria dichotoma* belongs to so called budding hydromedusae, which live along the coasts or in tidepools and have extraordinary feature of reproducing both sexually and asexually. Asexual reproduction of medusae (budding) allows them to reach high reproductive rates, and in turn high population densities. Conversely to the expectation, *Eleutheria dichotoma* is found always in low densities in the wild suggesting that populations may be regulated by different environmental factors, like salinity and temperature, because they directly affect growth and reproduction of marine organisms.

In five separate experiments I investigated the role of the two abiotic environmental factors (salinity and temperature) and one biotic factor (population density) in the reproduction and survival of *Eleutheria dichotoma*. I used samples of laboratory-cultured individuals

originating from a Banyuls-sur-Mer (southern France) collected several decades ago. I expected to find that coping with environmental stress should affect resource allocation patterns determining life-history strategies of growth, survival and reproduction. Particularly, I expected different responses to stress by medusae and hydroid colonies and significant effects of environment experienced by hydroids on performance of medusae.

In the first experiment (Experiment I, described in Chapters 2.4.1 and 3), hydroid colonies and medusae were exposed to different salinities (25, 35, and 45). Medusae were collected directly from colonies, and then reared for three generations, each obtained through asexual reproduction (budding medusa buds). I found that the salinity levels experienced by the hydroid colonies had relatively minor effects on life histories of medusae, such as initial size, maturity, budding medusa buds, larvae production, and survival. In contrast, salinity experienced directly by the medusae influenced their survival and reproduction. Low salinity conditions increased asexual reproduction (earlier onset and higher rates of budding) at the cost of decreased sexual reproduction (delayed onset and lower rates of larvae production). At medium salinity, decreasing budding rates across generations were accompanied by increasing larvae production rates. In contrast, at low salinity, larvae production rates decreased with each generation. Medusae reared at low salinity matured sexually later than medusae from medium salinity.

In the second experiment (Experiment II divided in Sub-experiment 1 and Sub-experiment 2, described in Chapter 4) I investigated the effects of temperature on reproduction and survival of the two clones of *Eleutheria dichotoma*. I conducted two separate experiments with low and medium (12°C, 23°C) as well as medium and high temperature (23°C, 28°C). The results of the two sub-experiments show that the medusae released from hydroid colony at 12°C and transferred to 23°C were bigger and matured sexually earlier than medusae that developed continuously at 23°C (control group). Though, they reproduced with lower rates. The effects

diminished in consecutive generations. Furthermore the medusae, which were released by hydroid colonies at temperature 28°C and were transferred to 23°C had higher sexual reproductive rates than controls in 23°C.

In the third experiment (Experiment III, described in Chapter 5) I investigated the effects of population density on the survival and reproductive strategies of a single clone of *Eleutheria dichotoma*. I found that sexual reproduction occurs with the highest rate at medium population densities. Increased sexual reproduction was associated with lower budding (asexual reproduction) and survival probability. Sexual reproduction may result in the production of motile larvae that can, in contrast to medusae, seek to escape unfavorable conditions by actively looking for better environments. The successful settlement of a larva results in starting the polyp stage, which is probably more resistant to environmental conditions. I found that most sexual and asexual reproduction occurred at the beginning of life following a very rapid process of maturation. The parametric models fitted to the mortality data showed that population density was associated with an increase in the rate of aging, an increase in the level of late-life mortality plateau, and a decrease in the hidden heterogeneity in individual mortality rates.

In the fourth experiment (Experiment IV described in Chapter 6) I investigated the combined effects of temperature (18, 23 and 28°C) and salinity (25, 35 and 45) on growth rate of hydroid colonies and production of medusae. The highest growth of the colonies, bigger sizes of hydranths (polyps), and the later onset of medusae production were observed in combination of low temperature (18°C) and low or medium salinities (25/35). The highest production of medusae was observed in medium temperature (23°C) and 25/35 salinities, which are conditions optimal for their sexual reproduction. In combination of high temperature (28°C) and medium salinity (35), the number of hydranths was generally higher, whereas the production of medusae was negligible, suggesting high investments in maintenance of the colony. The resistance of hydroid colonies and medusae to environmental stress was measured

by expression of heat shock proteins. Analysis of the samples showed that hydroid colonies might have more efficient mechanism protecting them from environmental stress than medusae.

Taking all things together, I showed that *Eleutheria dichotoma* have plastic life history strategies in response to different environmental factors. While hydroid colonies thrive at low temperatures and maintain local population at high temperatures, medusae shift their reproductive modes allowing rapid propagation (through budding medusa buds), or dispersal and escape from unfavorable environment (through production of planulae larvae). The dispersal and survival of these species is still possible even in extreme environmental conditions, where polyps serve as a reservoir for the genet. Furthermore, extraordinary variation in individual life histories open an opportunity for bed-hedging strategy. Clearly, *Eleutheria dichotoma* have strong adaptive potential to cope with variable environmental conditions as a result of the climate change.

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11 Supplementary materials

11.1 Pictures and figures

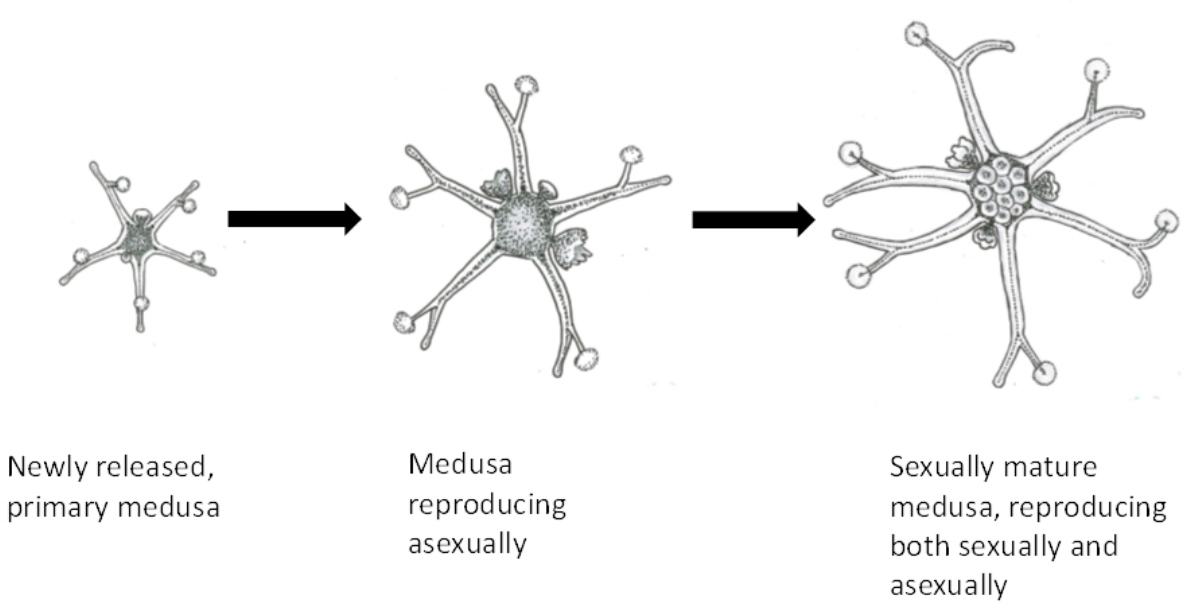


Figure A1 Medusae of *Eleutheria dichotoma* at different stages of development. Medusae that detached from hydroid colonies (primary medusae) first grow and then initiate asexual reproduction. After 2 – 4 weeks they mature sexually (embryos develop in a brood pouch placed on an aboral side of umbrella). Planula larvae are released through tearing of umbrella.

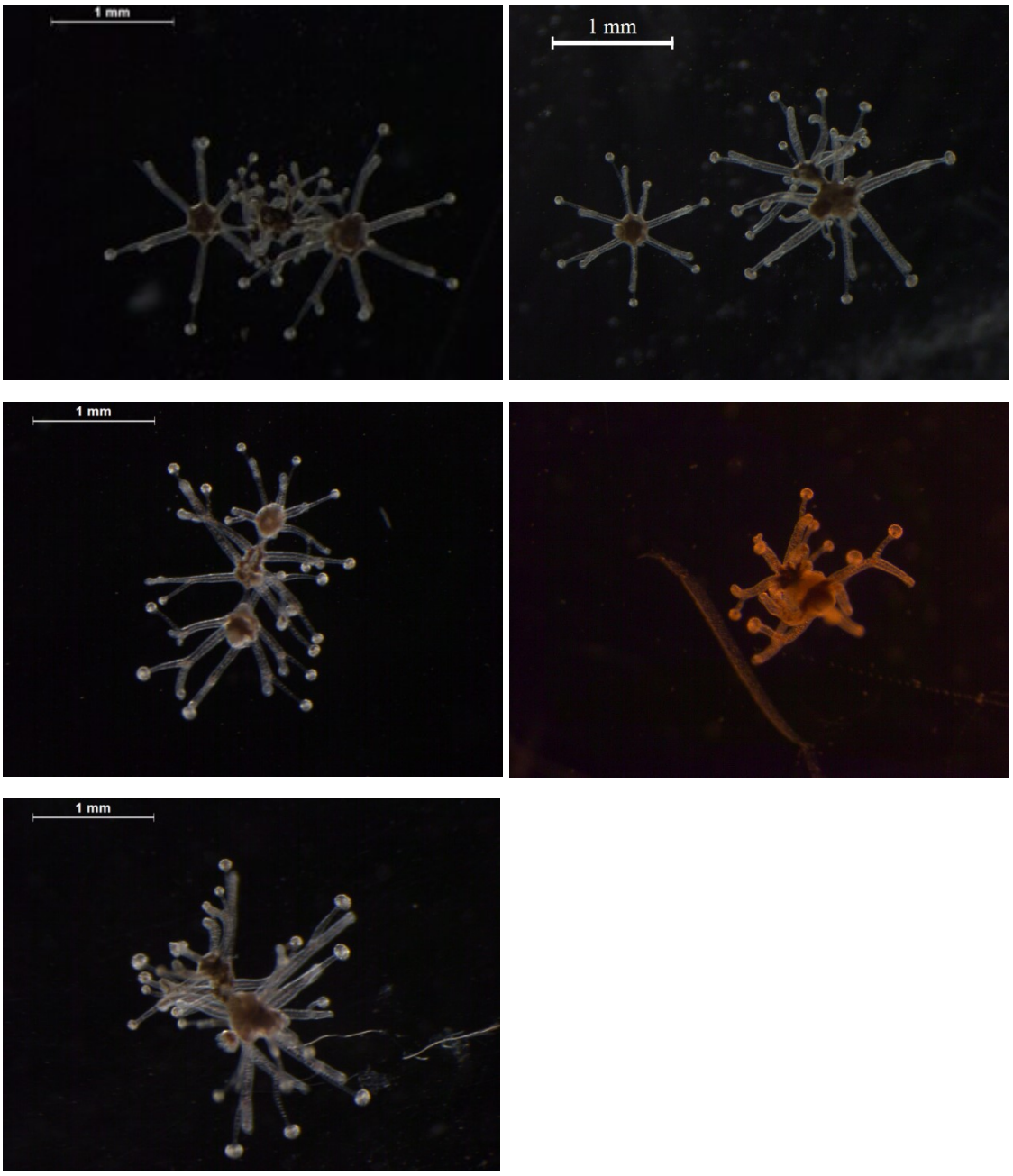
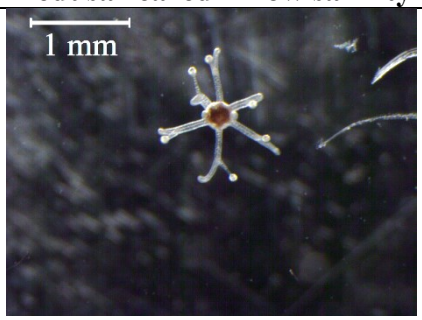
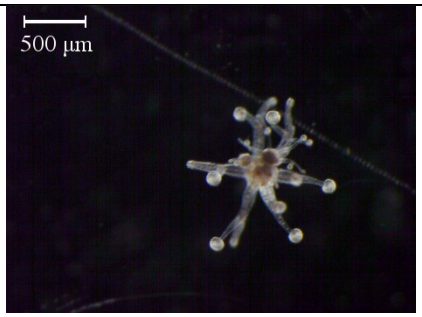
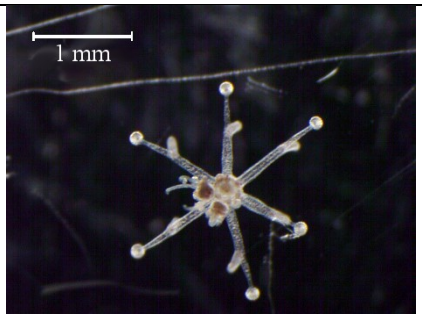
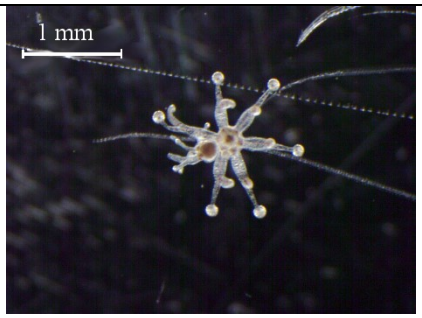
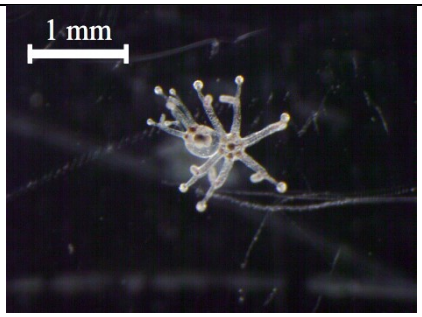
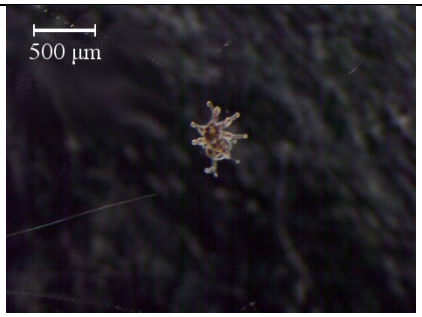
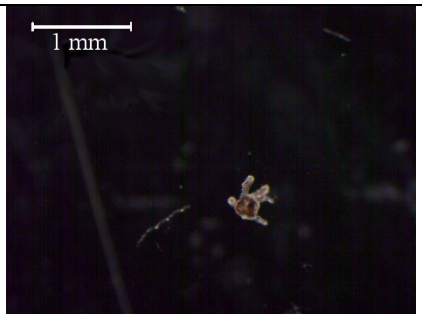
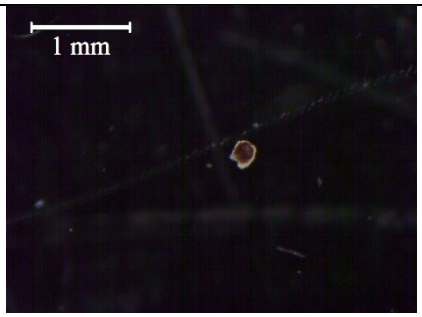
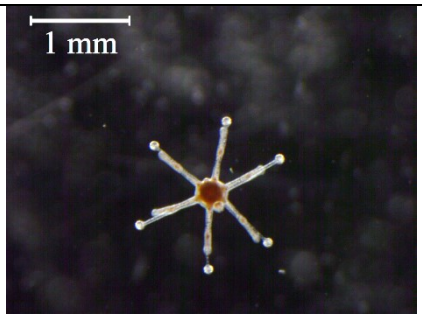
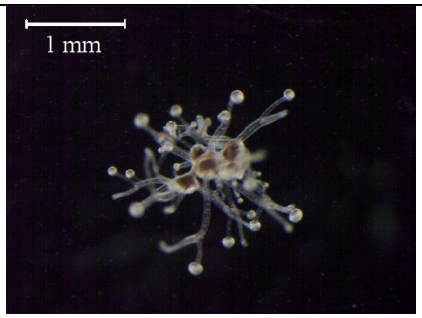
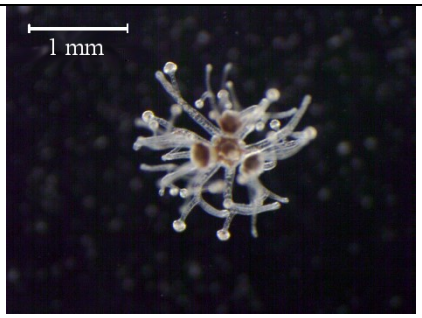


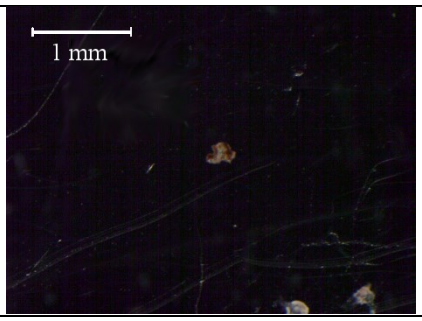
Figure A2. Degenerating medusae with overgrowing them buds. In many cases degenerating medusa was absorbed by a bigger bud.

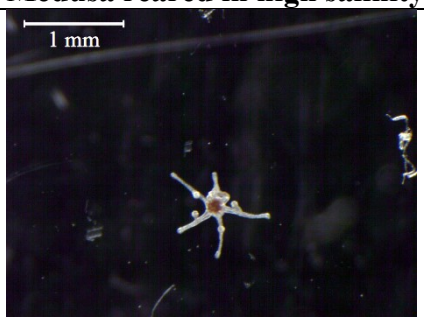
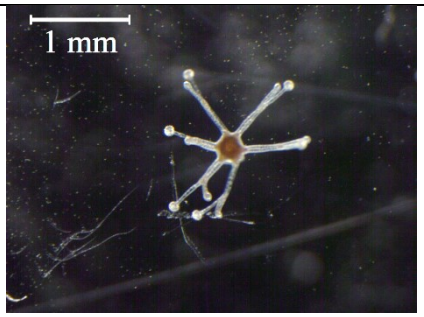
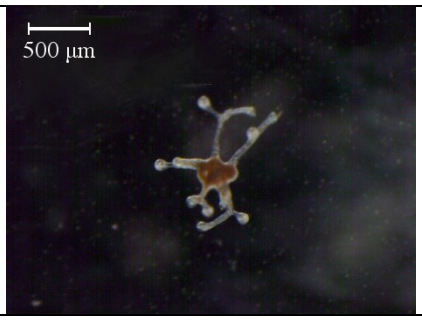
Figure A3. Morphological development of medusae at different salinities. Pictures show morphological changes over the life of one individual.

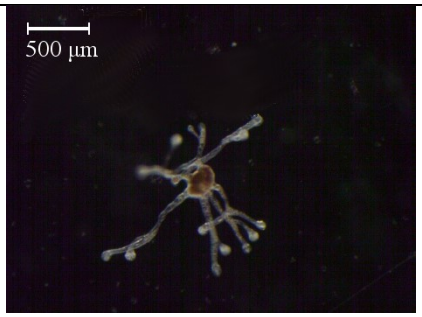
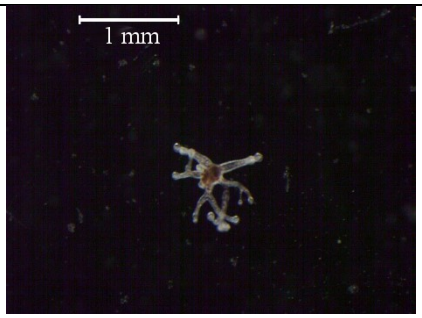
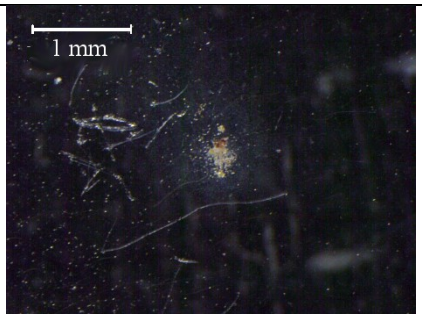
Date	Medusa reared in low salinity (25)
5 Nov 2014 (1 day old)	
26 Nov 2014 (3 week old)	
3 Dec 2014 (4 week old)	
8 Dec 2014	

18 Dec 2014 (1,5 month old)	
2 Jan 2015 (2 month old)	
7 Jan 2015 (2 month and 5 days old)	
12 Jan 2015 (dead, dissolving tissue)	

Date	Medusa reared in medium salinity (35)
5 Nov 2014 (1 day old)	
17 Nov 2014 (2 weeks old)	
26 Nov 2014 (3 weeks old)	
8 Dec 2014 (1 month old)	
17 Dec 2014 (1.5 month old)	

30 Dec 2014 **(2 months old)**	
9 Jan 2015 **(dead, dissolving tissue)**	

Date	**Medusa reared in high salinity (45)**
30 Oct 2014 **(1 day old)**	
5 Nov 2014 **(1 week old)**	
10 Nov 2014 **(1.5 week old)**	

14 Nov 2014 (2 weeks old)	
19 Nov 2014 (2.5 weeks old)	
11 Dec 2014 (1.5 month old; dead, dissolved tissue)	

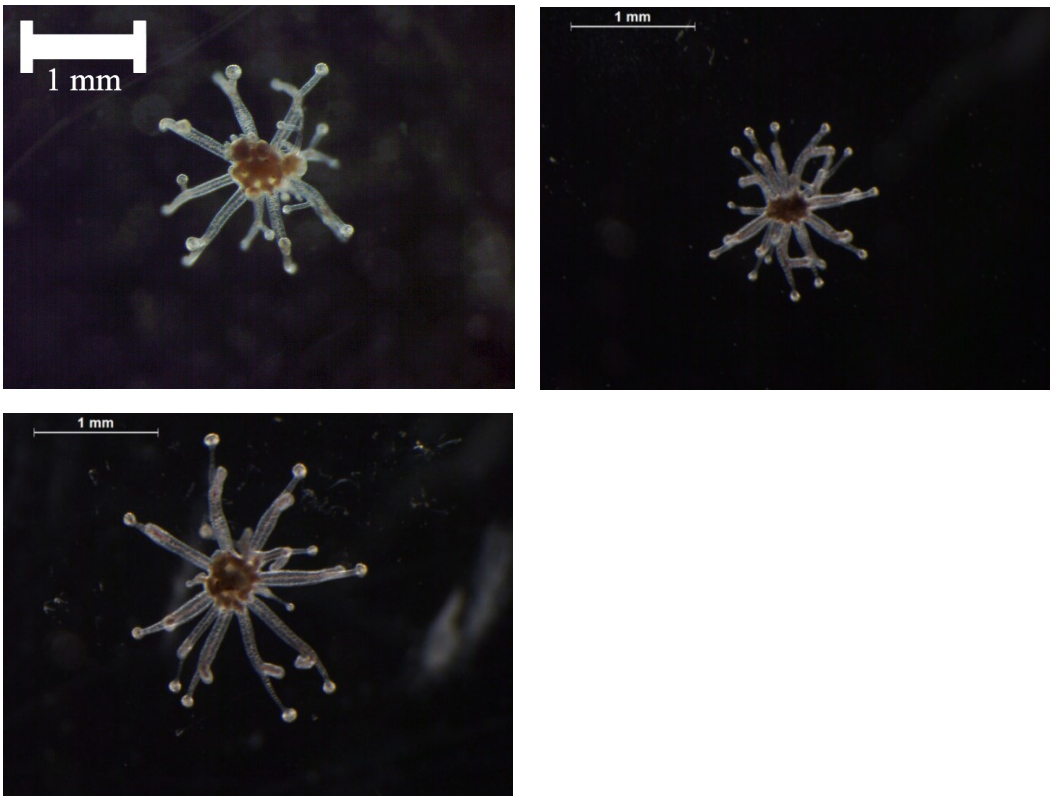


Figure A4 Medusae reared at temperature 28°C; The production of buds was impaired, instead medusae produced many additional tentacles.

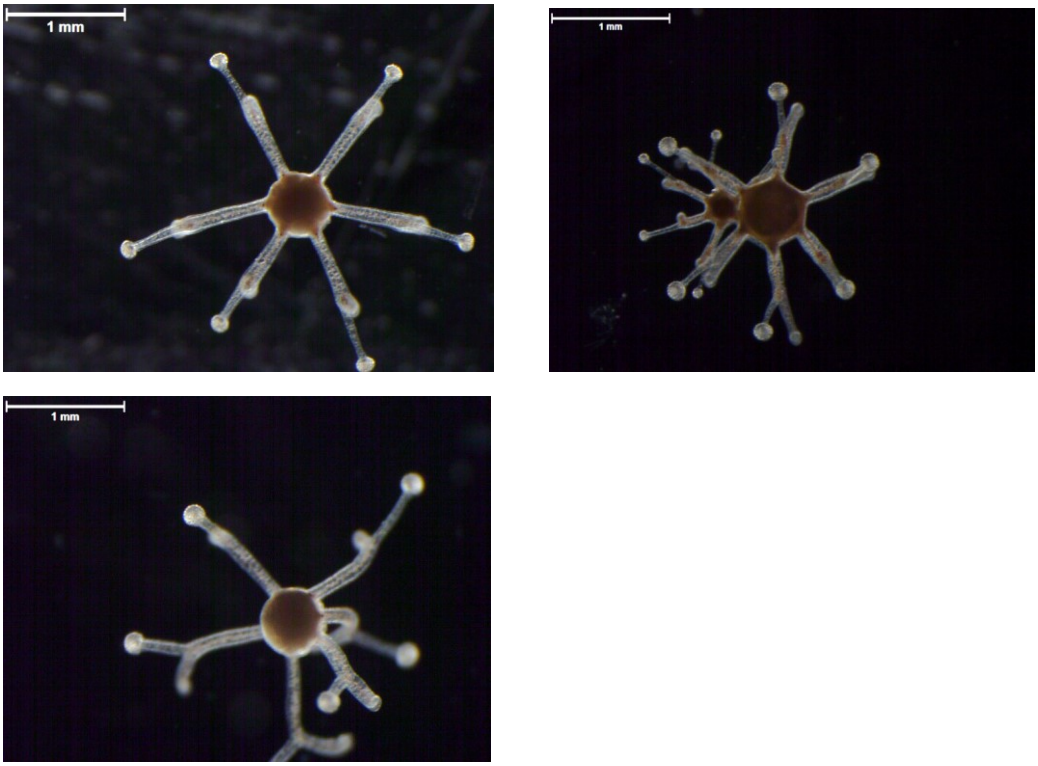


Figure A5 Medusae reared at temperature 12°C; Medusae are larger than medusae from other regimes.

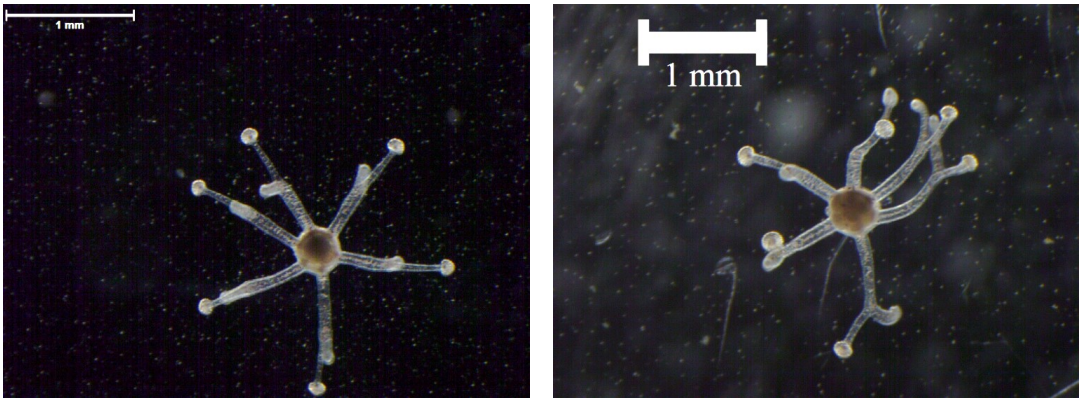


Figure A6 Mature medusae reared at temperature 23°C. Developing embryos are visible in umbrella.

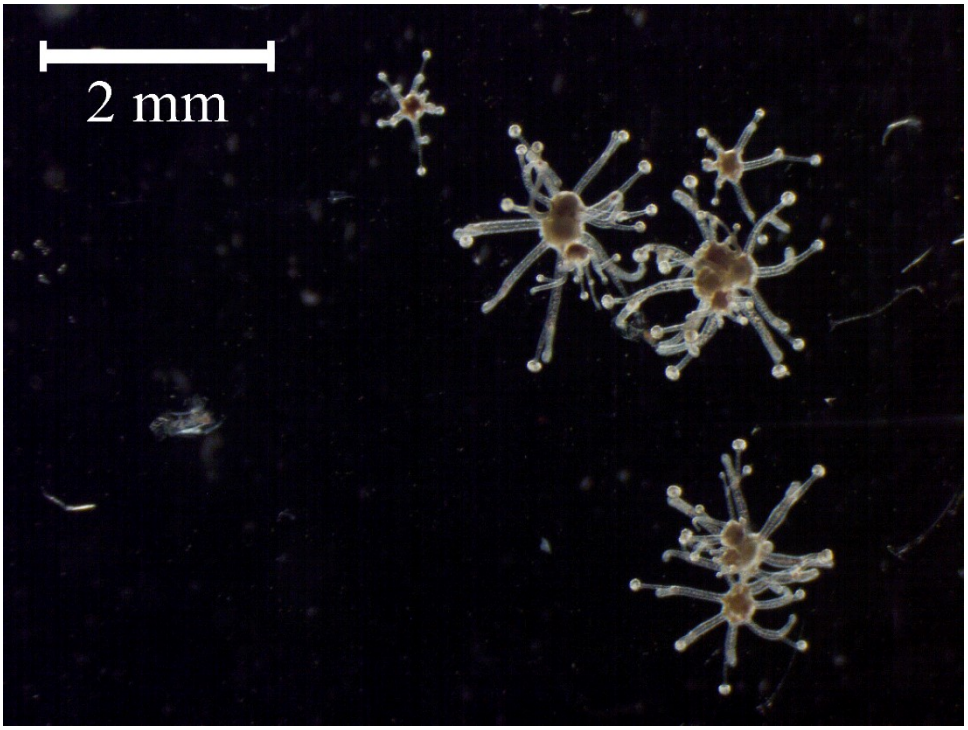


Figure A7. Three experimental medusae and two released buds (the buds are smaller than the medusa mothers)

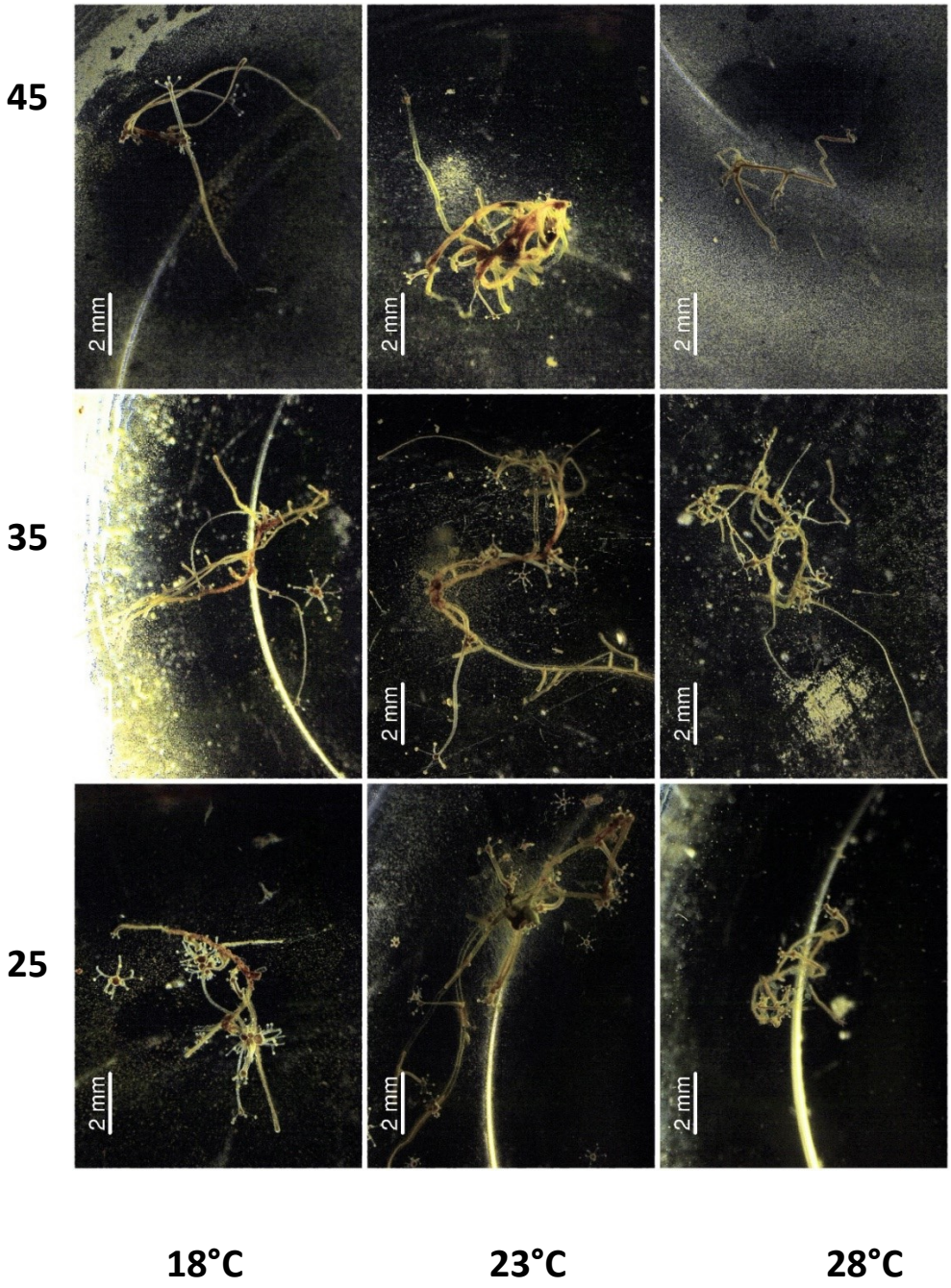


Figure A8. Exemplary hydroid colonies at similar age (4-5 weeks) maintained in different temperatures and salinities. The colonies differed morphologically depanding on culturing conditions. In low temperature and low salinity, hydranths were bigger. In high temperature, hydrorhiza (a system of stolons) was covered with a transparent and fragile perisarc. The colonies reared at high temperature produced many hydranths, which usualy did not develop medusa buds.

11.2 Tables

Table A1. Test of the effects of the salinity experienced by hydroid colonies on survival of medusae. Salinity experienced by the hydroid colonies have no significant effect on the survival of the medusae.

Group	Compared	Df	Log-rank stat	Log-rank p	Holm's Log-rank p	Gehan-Breslow stat	Gehan-Breslow p	Holm's Gehan-Breslow p
M25, G1	P25 and P35	1	2.13	0.0334	0.2338	1.37	0.172	1
M35, G1	P25, P35 and P45	2	0.03	0.9834	1	0.88	0.6434	1
M45, G1	P25 and P35	1	1.17	0.242	1	1.27	0.2032	1
M25, G2	P25 and P35	1	-0.88	0.3813	1	-0.57	0.5718	1
M35, G2	P25 and P35	1	-1.5	0.1325	0.795	-1.18	0.2378	1
M25, G3	P25 and P35	1	0.13	0.8975	1	-0.24	0.8093	1
M35, G3	P25 and P35	1	-1.02	0.3082	1	-0.1	0.9183	1
M25, pooled generations	P25 and P35	1	0.29	0.773	0.9674	0.05	0.9613	0.9613
M35, pooled generations	P25 and P35	2	1.45	0.4837	0.9674	2.19	0.3349	0.6698

11.3 Coeficients and significance of the fitted models

Table A2. Results of the GAMM model fited to the stolon length data.

Fixed terms	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	0.9042	0.2717	3.33	0.0009
Temp (23C)	-0.2877	0.3057	-0.94	0.3466
Temp (28C)	-0.2803	0.3321	-0.84	0.3987
Sal (35)	-0.1233	0.2556	-0.48	0.6295
Sal (45)	-1.4708	0.2814	-5.23	0.0000
Temp (23C) : Sal (35)	0.5451	0.3545	1.54	0.1242
Temp (28C) : Sal (35)	0.4210	0.4140	1.02	0.3092
Temp (23C) : Sal (45)	1.8293	0.3750	4.88	0.0000
Temp (28C) : Sal (45)	1.5495	0.4365	3.55	0.0004
Smooth terms	**edf**	**Ref.df**	**F**	**Pr(>F)**
s(Age)	10.7438	19	433.35	0.0000
ti(Age) : Temp (23C)	3.3203	18	89.00	0.0000
ti(Age) : Temp (28C)	4.2303	19	201.32	0.0000
ti(Age) : Sal (35)	0.0000	19	0.00	0.7992
ti(Age) : Sal (45)	0.0000	17	0.00	0.7327
ti(Age) : Temp (18C) : Sal (25)	0.0003	13	0.00	1.0000
ti(Age) : Temp (23C) : Sal (25)	0.0000	16	0.00	0.8774
ti(Age) : Temp (28C) : Sal (25)	3.1848	19	53.24	0.0000
ti(Age) : Temp (18C) : Sal (35)	0.0463	13	0.05	0.3022
ti(Age) : Temp (23C) : Sal (35)	0.0002	12	0.00	0.3918
ti(Age) : Temp (28C) : Sal (35)	0.0000	14	0.00	0.6687
ti(Age) : Temp (18C) : Sal (45)	0.9757	9	58.14	0.0000
ti(Age) : Temp (23C) : Sal (45)	0.0000	12	0.00	0.6178
ti(Age) : Temp (28C) : Sal (45)	0.3340	9	0.88	0.2172
Smooth random terms	**edf**	**Ref.df**	**F**	**Pr(>F)**
s(Colony)	18.6877	27	1274.48	0.0012
s(Colony, Age)	13.6070	36	932.08	0.0342

Table A3. Results of the GAMM model fited to the number of branches data.

Fixed terms	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	-2.9359	2.3567	-1.25	0.2128
Temp (23C)	0.7339	2.3927	0.31	0.7591
Temp (28C)	2.4642	2.3747	1.04	0.2994
Sal (35)	2.2469	2.4844	0.90	0.3658
Sal (45)	1.8494	2.6196	0.71	0.4802
Temp (23C) : Sal (35)	-1.8152	2.5370	-0.72	0.4743
Temp (28C) : Sal (35)	-2.9257	2.5165	-1.16	0.2450

Temp (23C) : Sal (45)	-0.9250	2.6371	-0.35	0.7258
Temp (28C) : Sal (45)	-4.4408	3.1925	-1.39	0.1642
Smooth terms	**edf**	**Ref.df**	**Chi²**	**Pr(>Chi²)**
s(Age)	0.0000	19	0.00	0.7774
ti(Age) : Temp (23C)	5.8275	19	60.39	0.0000
ti(Age) : Temp (28C)	7.2984	19	42.87	0.0000
ti(Age) : Sal (35)	2.9565	19	9.81	0.0019
ti(Age) : Sal (45)	0.0000	18	0.00	0.8587
ti(Age) : Temp (18C) : Sal (25)	8.3365	17	48.63	0.0000
ti(Age) : Temp (23C) : Sal (25)	0.7553	17	2.94	0.0506
ti(Age) : Temp (28C) : Sal (25)	0.3169	18	0.38	0.2409
ti(Age) : Temp (18C) : Sal (35)	10.4342	19	31.56	0.0000
ti(Age) : Temp (23C) : Sal (35)	1.6863	19	3.96	0.0341
ti(Age) : Temp (28C) : Sal (35)	0.0000	15	0.00	1.0000
ti(Age) : Temp (18C) : Sal (45)	7.8348	13	25.82	0.0007
ti(Age) : Temp (23C) : Sal (45)	0.0000	15	0.00	0.7983
ti(Age) : Temp (28C) : Sal (45)	4.0652	18	6.74	0.1157
Smooth random terms	**edf**	**Ref.df**	**Chi²**	**Pr(>Chi²)**
s(Colony)	0.0011	27	0.00	0.7226
s(Colony, Age)	12.3366	36	21.43	0.0122

Table A4. Results of the GAMM model fitted to the number of hydranths data.

Fixed terms	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	1.0078	0.1697	5.94	0.0000
Temp (23C)	-0.4283	0.2382	-1.80	0.0722
Temp (28C)	-0.6228	0.2186	-2.85	0.0044
Sal (35)	-0.8666	0.2230	-3.89	0.0001
Sal (45)	-1.3374	0.2345	-5.70	0.0000
Temp (23C) : Sal (35)	1.0211	0.3218	3.17	0.0015
Temp (28C) : Sal (35)	0.9924	0.3211	3.09	0.0020
Temp (23C) : Sal (45)	1.2433	0.3386	3.67	0.0002
Temp (28C) : Sal (45)	1.4508	0.3283	4.42	0.0000
Smooth terms	**edf**	**Ref.df**	**Chi²**	**Pr(>Chi²)**
s(Age)	7.9161	11	361.67	0.0000
ti(Age) : Temp (23C)	0.6835	10	2.65	0.0456
ti(Age) : Temp (28C)	0.0001	11	0.00	0.5547
ti(Age) : Sal (35)	0.6892	11	2.80	0.0528
ti(Age) : Sal (45)	0.0000	11	0.00	0.6474
ti(Age) : Temp (18C) : Sal (25)	0.0002	11	0.00	0.3888
ti(Age) : Temp (23C) : Sal (25)	3.1640	11	38.62	0.0000
ti(Age) : Temp (28C) : Sal (25)	0.0002	11	0.00	0.7083
ti(Age) : Temp (18C) : Sal (35)	0.0000	11	0.00	0.7062

ti(Age) : Temp (23C) : Sal (35)	0.9082	11	13.34	0.0001
ti(Age) : Temp (28C) : Sal (35)	0.7851	10	4.67	0.0208
ti(Age) : Temp (18C) : Sal (45)	0.0001	10	0.00	0.4561
ti(Age) : Temp (23C) : Sal (45)	0.5969	11	0.99	0.1562
ti(Age) : Temp (28C) : Sal (45)	0.0000	11	0.00	1.0000
Smooth random terms	**edf**	**Ref.df**	**Chi^2**	**$Pr(>Chi^2)$**
s(Colony)	9.3796	27	117.44	0.1935
s(Colony, Age)	16.2522	36	255.04	0.0019

Table A5. Results of the GAMM model fitted to the medusae production rate data.

Fixed terms	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	-4.2474	0.6446	-6.59	0.0000
Temp (23C)	0.5887	0.4705	1.25	0.2109
Temp (28C)	-4.9569	0.8409	-5.89	0.0000
Sal (35)	0.1589	0.4237	0.37	0.7077
Sal (45)	-3.3688	0.9781	-3.44	0.0006
Temp (23C) : Sal (35)	-0.4005	0.5721	-0.70	0.4839
Temp (28C) : Sal (35)	1.8511	1.2826	1.44	0.1489
Temp (23C) : Sal (45)	2.8355	1.0252	2.77	0.0057
Temp (28C) : Sal (45)	4.9090	1.3910	3.53	0.0004
Smooth terms	**edf**	**Ref.df**	**Chi^2**	**$Pr(>Chi^2)$**
s(Age)	4.4528	11	49.81	0.0000
ti(Age) : Temp (23C)	7.2184	11	60.74	0.0000
ti(Age) : Temp (28C)	0.0000	11	0.00	0.5732
ti(Age) : Sal (35)	0.0000	11	0.00	0.6394
ti(Age) : Sal (45)	0.0000	11	0.00	0.8298
ti(Age) : Temp (18C) : Sal (25)	2.0996	11	6.07	0.0309
ti(Age) : Temp (23C) : Sal (25)	0.0001	11	0.00	0.8410
ti(Age) : Temp (28C) : Sal (25)	0.0000	11	0.00	1.0000
ti(Age) : Temp (18C) : Sal (35)	0.0002	11	0.00	0.8789
ti(Age) : Temp (23C) : Sal (35)	2.3441	11	8.37	0.0151
ti(Age) : Temp (28C) : Sal (35)	5.8883	11	66.84	0.0000
ti(Age) : Temp (18C) : Sal (45)	0.9373	10	13.14	0.0002
ti(Age) : Temp (23C) : Sal (45)	0.0001	11	0.00	0.5320
ti(Age) : Temp (28C) : Sal (45)	0.9736	11	1.91	0.1427
Smooth random terms	**edf**	**Ref.df**	**Chi^2**	**$Pr(>Chi^2)$**
s(Colony)	0.0010	27	0.00	0.6359
s(Colony, Age)	12.9521	36	26.81	0.0019

11.4 Multiple comparisons

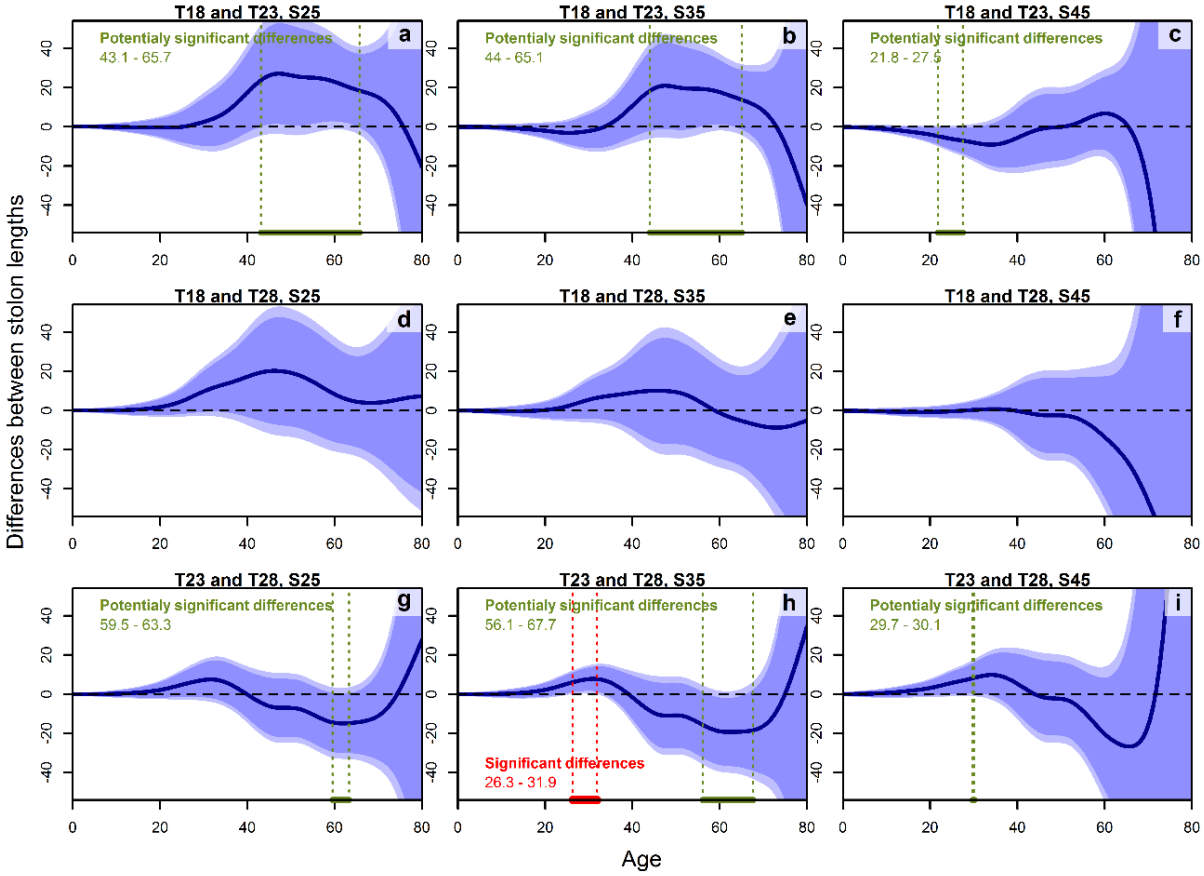


Figure A7.1. Differences in length of the stolon between different pairs of temperature.

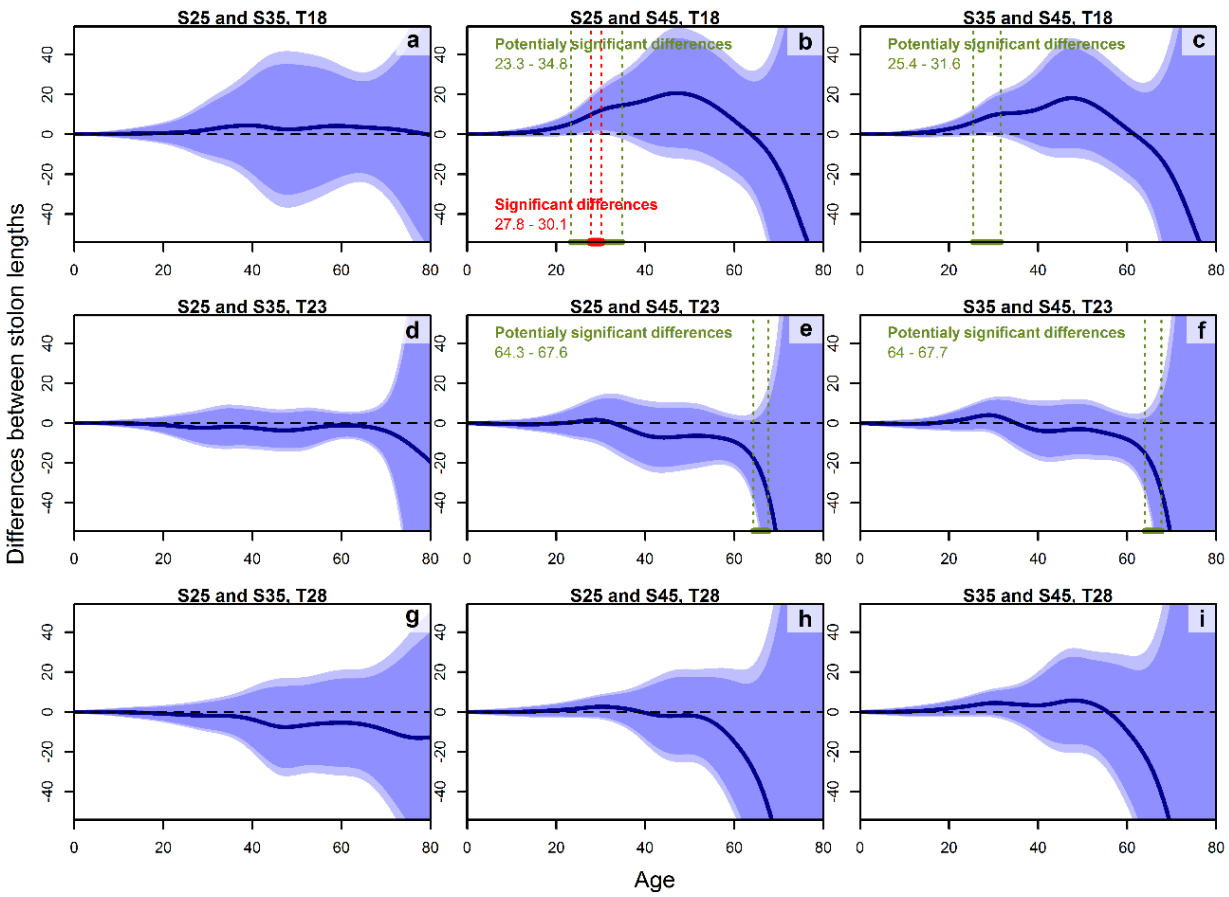


Figure A7.2. Differences in length of the stolon between different pairs of salinity.

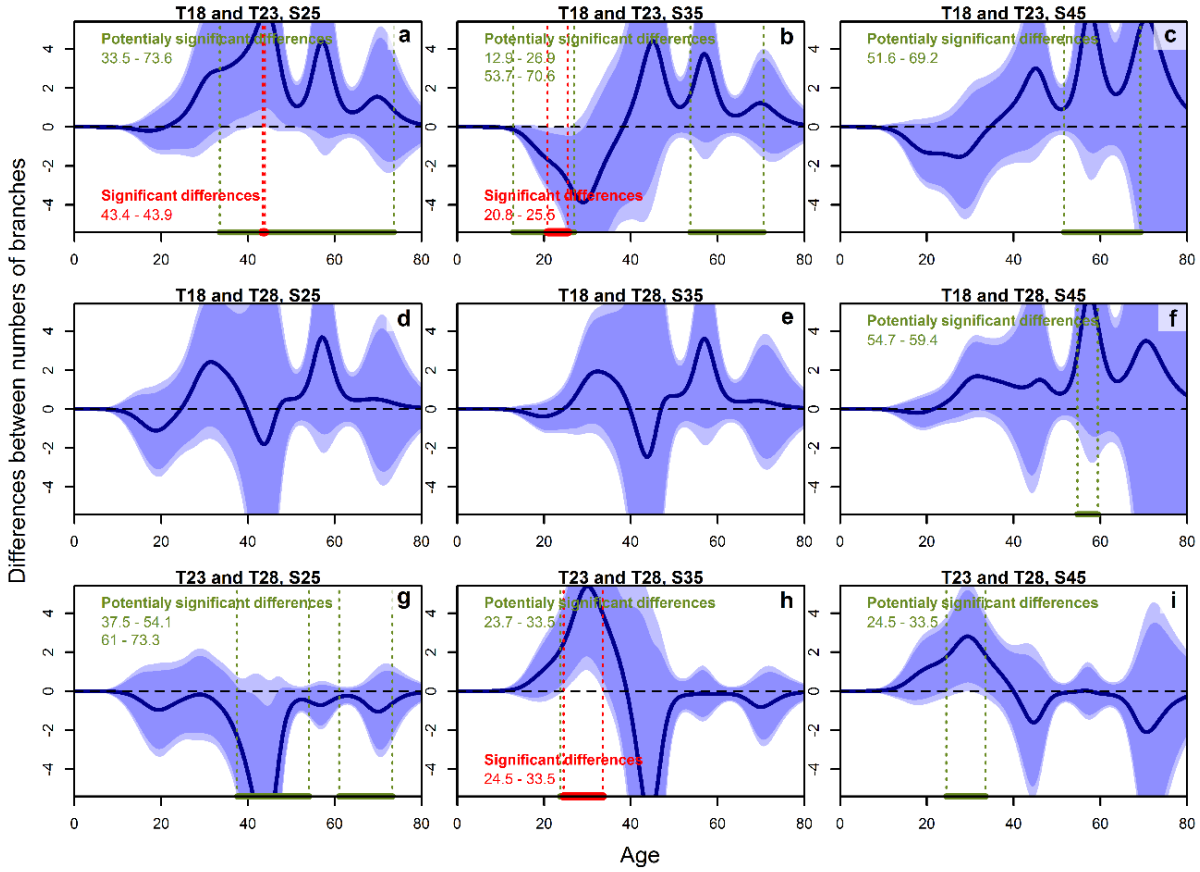


Figure A8.1. Differences in the number of stolon branches between different pairs of temperature.

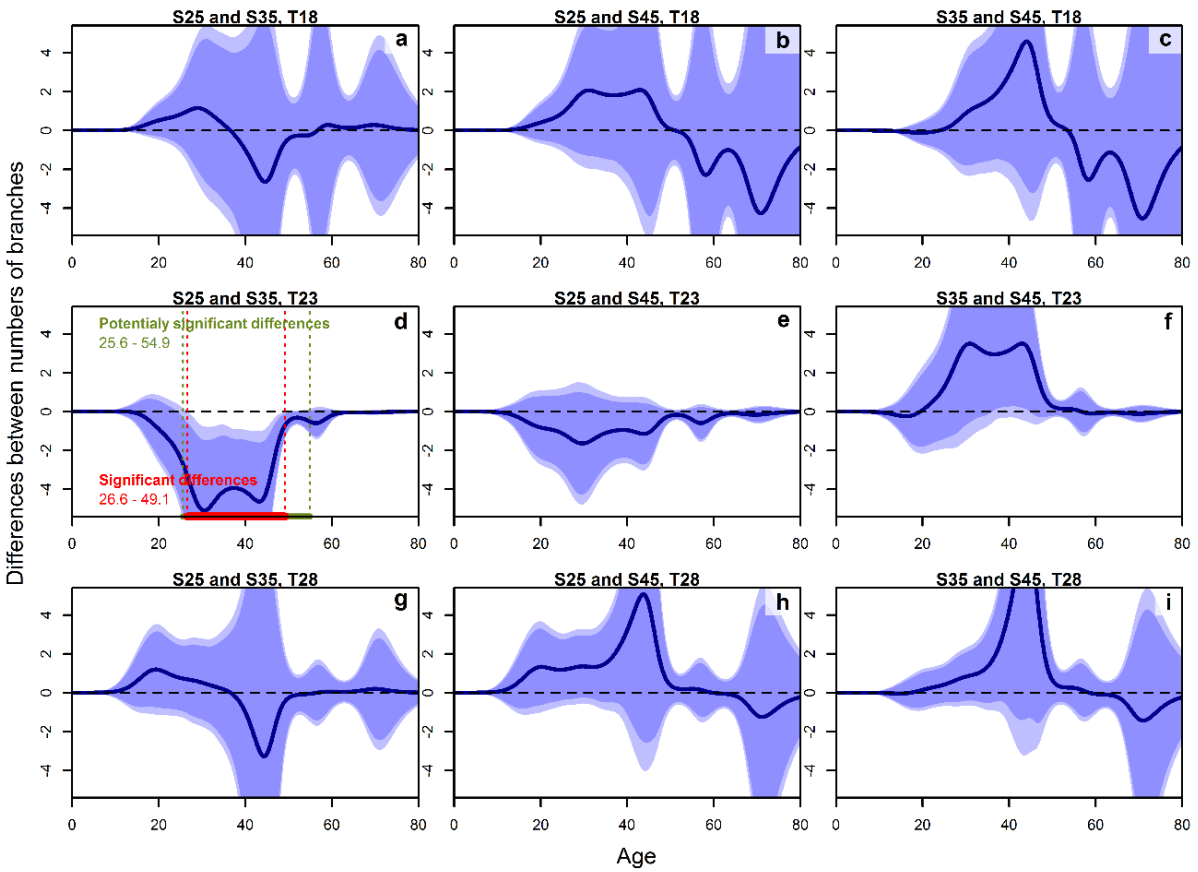


Figure A8.2. Differences in the number of stolonal branches between different pairs of salinity.

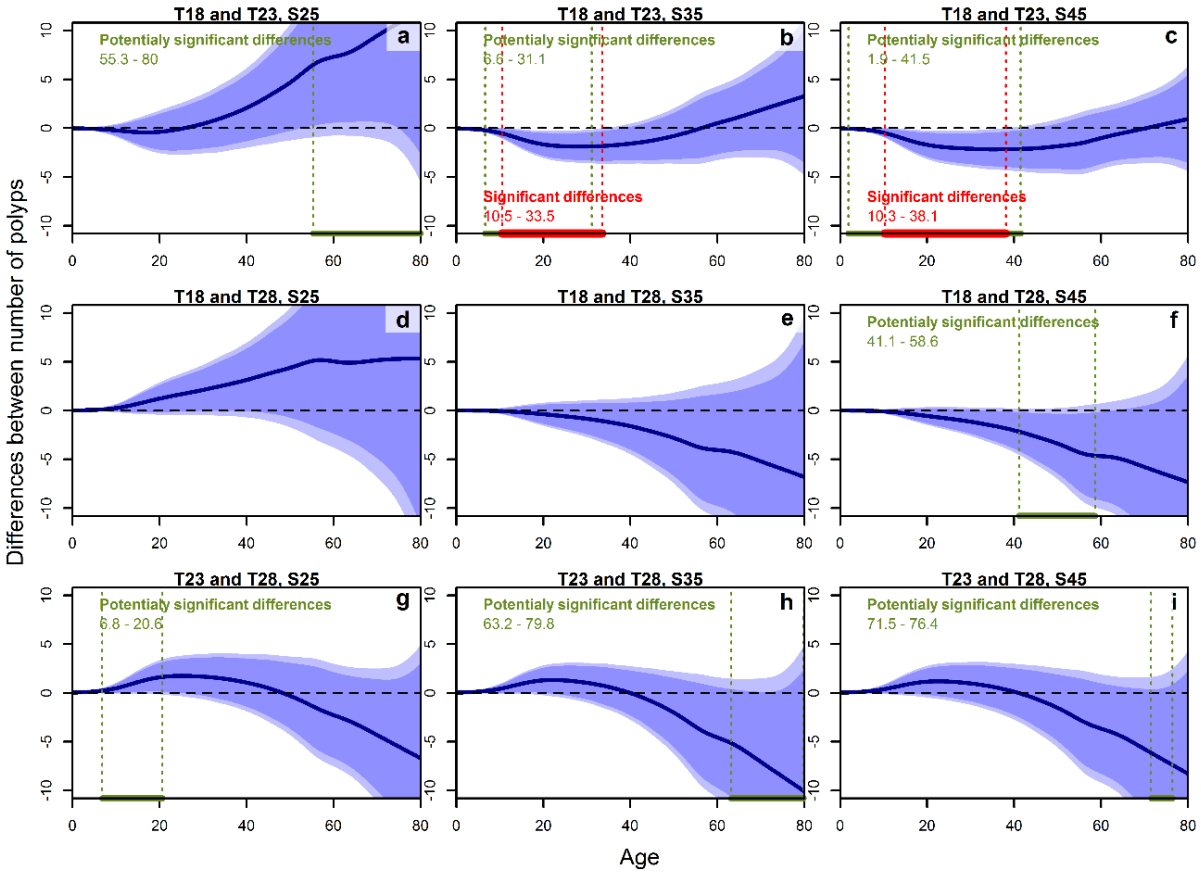


Figure A9.1. Differences in the number of hydranths between different pairs of temperature.

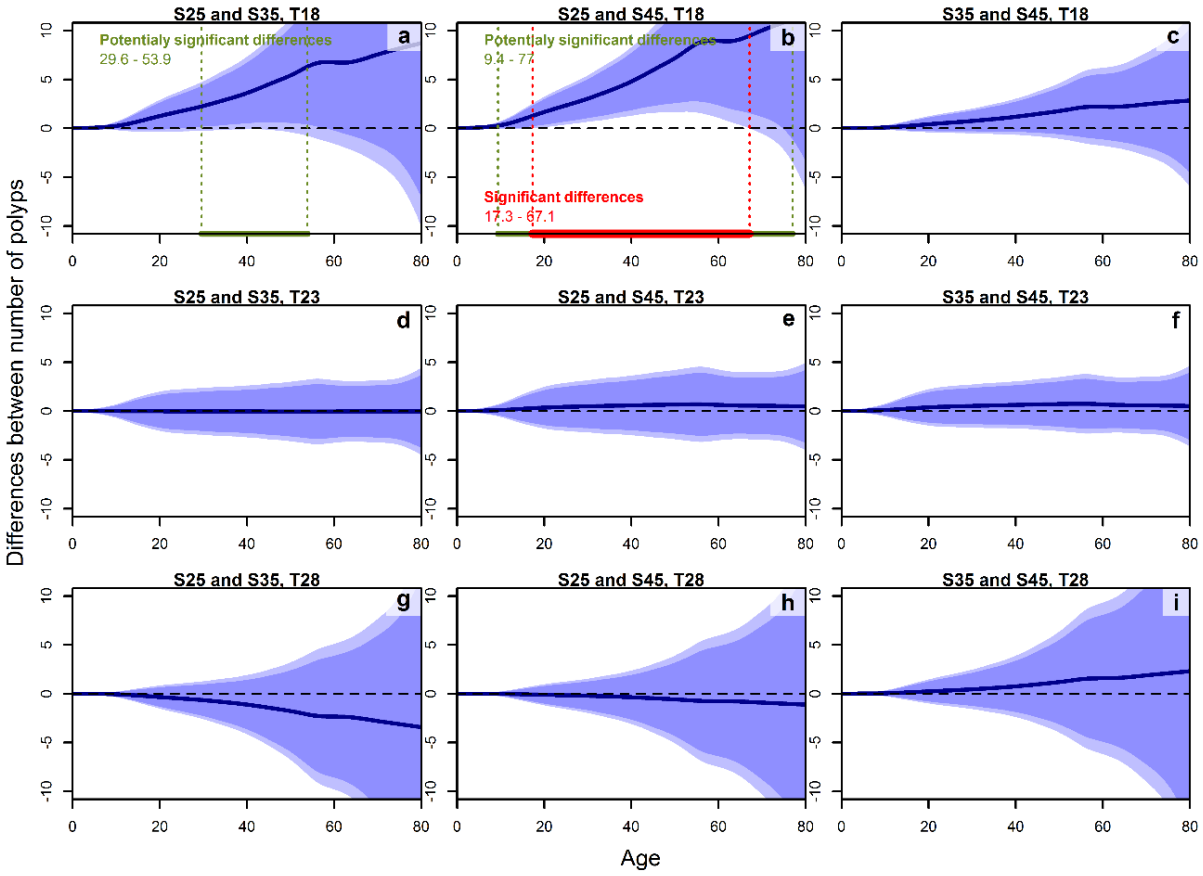


Figure A9.2. Differences in the number of hydranths between different pairs of salinity.

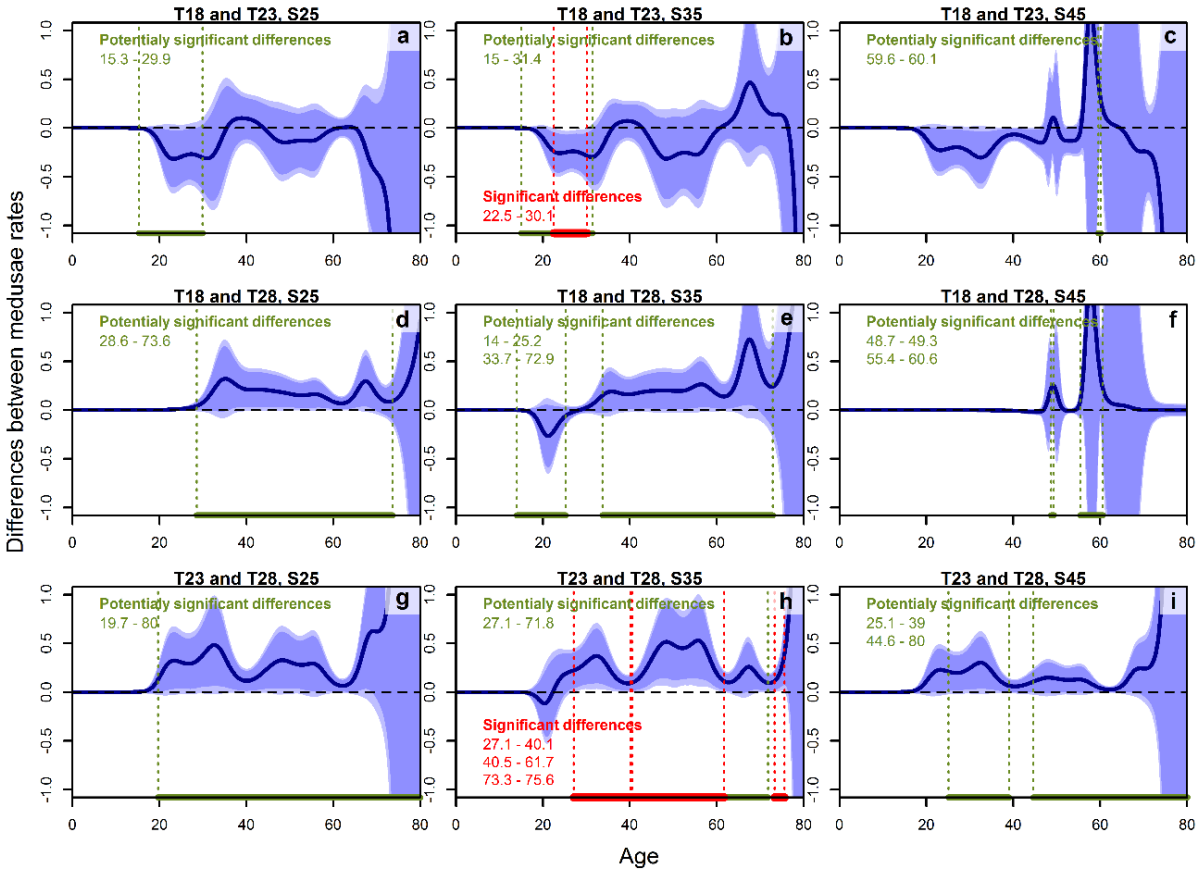


Figure A10.1. Differences in the medusae production rate between different pairs of temperature .

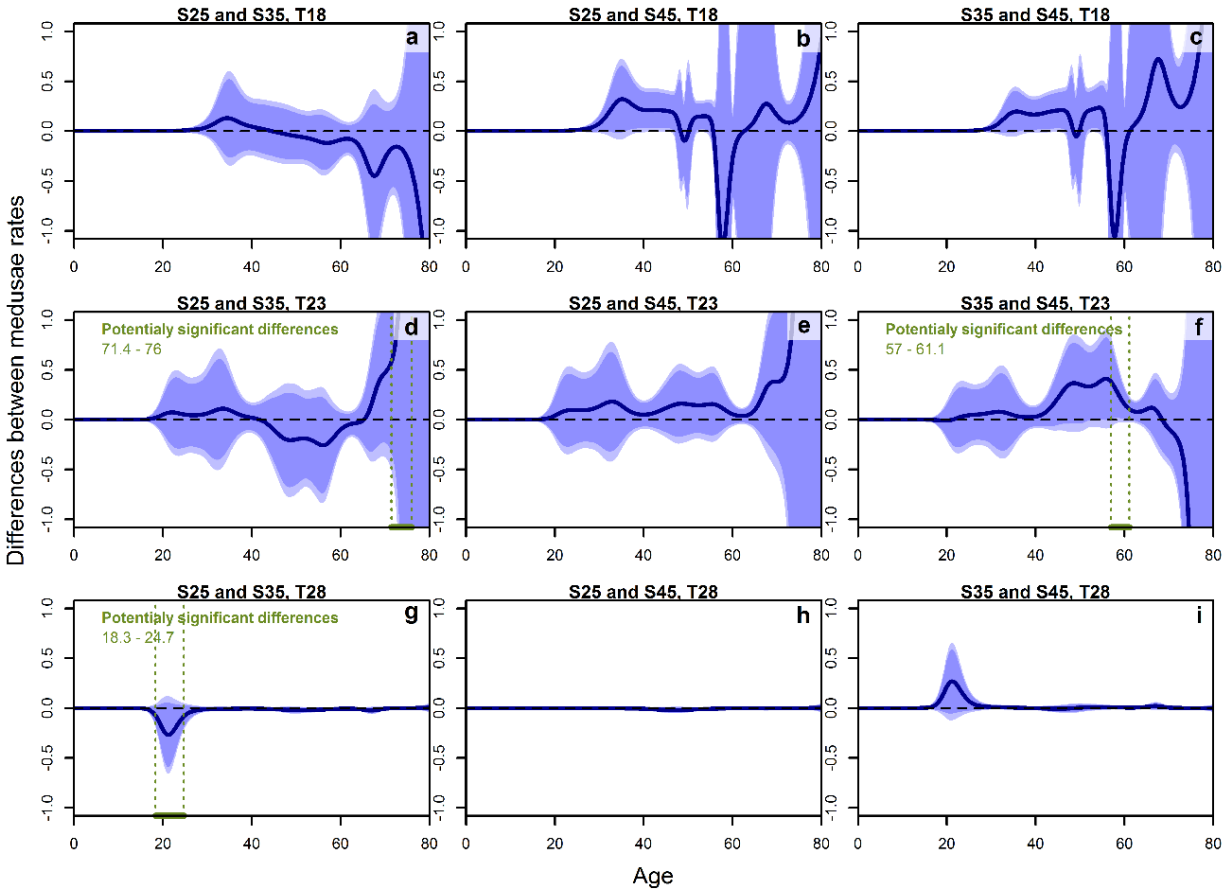


Figure A10.2. Differences in the medusae production rate between different pairs of salinity. At medium temperature, similar fluctuations at da 40 and 60 are observed

Curriculum Vitae

Work Experience

01/2018 – 06/2019	**Guest Researcher** at the Max Planck Institute for Demographic Research;
03/2017 – 12/2017	**Research Scientist** at the Max Plank Institute;
03/2012 – 02/2017	**PhD Student** at the Max Planck Institute; Laboratory of Evolutionary
10/2006 – 04/2008	**Senior Laboratory Assistant** in Veterinary Inspectorate, Department of Veterinary Hygiene, Cracow, Poland Laboratory detecting Bovine Spongiform Encephalopathy and Scrapie Diseases
10/2004 – 09/2006	**Junior Laboratory Assistant** in Veterinary Inspectorate, Cracow, Poland; Department of Veterinary Hygiene, Cracow, Poland Laboratory detecting Bovine Spongiform Encephalopathy and Scrapie Diseases
06/2004 – 09/2004	**Technical Assistant** in Veterinary Inspectorate, Cracow, Poland; Department of Veterinary Hygiene, Cracow, Poland Laboratory detecting Bovine Spongiform Encephalopathy and Scrapie Diseases
12/2003 – 05/2004	**Trainee** in Veterinary Inspectorate Department of Veterinary Hygiene, Cracow, Poland Laboratory detecting Bovine Spongiform Encephalopathy and Scrapie Diseases

Academic Education

15/07/2017 – until now	**PhD Student** at the Rostock University
03/2012 – 02/2017	**PhD Student** at Max Planck Institute for Demographic Research
10/1998 – 05/2003	**Master of Science**, Faculty of Biology, Institute of Environmental Sciences, Population Ecology Lab, Jagiellonian University, Cracow, Poland

Professional Training

02/2018 – 03/2018	Assistant of Quality Manager (alfa-training, Rostock)

Skills

Language skills	Polish (native speaker) English (excellent) Deutsch (Goethe Zertifikat C1) Italienisch (Basic, Level A2)
Driving licence	PKW B
Data analysis	Statistica (excellent), R Core
Computer skills	MS Office (excellent)

Activities and Hobbies

Music (playing Viola in Youth Symphonic Orchestra of Rostock) and drawning

Conferences and Posters

04/2016, Warsaw, Poland	Warsaw University Presentation: *Reproductive strategy and survival in different environmental conditions in hydromedusa Eleutheria dichotoma – Summary of the PhD Project*
9/2015, Koszalin, Poland	XXIII Konferencja Hydrobiologów Polskich Presentation: Wpływ zasolenia na rozmnażanie i śmiertelność hydromeduzy *Eleutheria dichotoma*
10/2014, Warsaw, Poland	Warsaw University Presentation: *Environmental influence on life history of Eleutheria dichotoma – a project for PhD Thesis*
9/2014 La Roche-en-Ardenne, Belgium	European Meeting of PhD Students in Evolutionary Biology Presentation: *Effects of environmental factors on reproduction and survival of hydromedusae Eleutheria dichotoma*
10/2013 Odense, Danmark	South Danmark University Poster: *Effects of temperature on reproduction and survival of medusae Eleutheria dichotoma*

Publications

23/03/2018	Dańko, A., Schaible, R., Pijanowska, J., & Dańko, M. J. (2018). Population density shapes patterns of survival and reproduction in *Eleutheria dichotoma (Hydrozoa: Anthoathecata*). *Marine Biology, 165*(3/48), 1-10. published online: http://hdl.handle.net/21.11116/0000-0004-7C67-E
23/12/2019	Dańko, A., Schaible, R., Dańko, M. J. Salinity effects on survival and reproduction of hydrozoan *Eleutheria dichotoma;* *Estuaries and Coasts*, 43:2, 360–374 (2020) DOI:10.1007/s12237-019-00675-2
Rejected	Temperature and salinity affect growth and reproduction in hydroid colonies of *Eleutheria dichotoma* (Hydrozoa: Anthoathecata: Cladonematidae)
In preparation	Temperature affects reproduction and survival of medusae in *Eleutheria dichotoma*

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Declaration of Authorship

I, the undersigned hereby, declare that this research thesis is my own original work and that all sources had been accurately reported and acknowledged, and that this document has not been previously, in its entirety or in part, submitted at any university in order to obtain academic qualifications.

Aleksandra Dańko Rostock, ……