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Ocean and Earth Science

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Abstract

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What Drives Jellyfish Population Cycles? Influence of Climate and Environment on the Complex Life Histories of Scyphozoans

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Jellyfish population cycles and bloom events occur at global, regional, and local scales. Understanding what causes these cycles now and in the future is a major question in jellyfish bloom research, because of the potential impacts on ecosystem function and services. Most bloom forming scyphozoan jellyfish have complex life histories involving a long-lived asexually reproducing benthic polyp and a sexually reproducing pelagic medusae. Environmental and climate factors affect each life stage, but we do not fully understand how these variables drive life stage transition, or how demographic differences in survival, growth and fecundity translate into visible jellyfish outbreaks. We undertook a comprehensive laboratory and field-based study of the physicochemical conditions that control survival, fecundity and phase transition of the different life stages of scyphozoan jellyfish. Through this research, we examine the effects of environmental drivers on jellyfish population cycles and life stage transition. Modifications to estuaries through the construction of barrages alter the natural dynamics of inhabitant species by controlling freshwater inputs into those systems, driving the presence and absence of medusae from estuaries. As well as this, we explore how environmental conditions translate into reproductive success or failure in temperate populations from the medusa to the polyp life stage, demonstrating that early polyp growth rates are strongly linked to their thermal environment and highlighting a potential marine heatwave event. We examine not only the effects of temperature and other climate drivers on scyphozoan jellyfish growth, survival and reproduction, but also whether epigenetic transgenerational effects can drive acclimation to warmer summer temperatures in the short term in the context of a warming ocean. No parental effects were observed in the first or second generation, and in the third generation the transgenerational effects of temperature were subtle and appeared most strongly in cooling scenarios. Finally, within the setting of anthropogenically-driven climate change, we demonstrate for the first time that *A. aurita* polyps require a minimum period of cooler temperatures to strobilate, contradicting claims that jellyfish populations will be more prevalent in warming oceans, specifically in the context of warmer winter conditions. To answer these questions, we chose the common, or moon jellyfish *Aurelia aurita* as our primary experimental organism. However, we expanded our research to other species to demonstrate how they may vary in both environment and response to forcing factors as compared to a 'typical' model species. This thesis highlights the importance of examining each population within the context of their environment, and advances our understanding of how the climate and environment affect jellyfish life stage transition.

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Research Thesis: Declaration of Authorship

Print name: **ALEXANDRA LOVERIDGE**

Title of thesis: **What Drives Jellyfish Population Cycles? Influence of Climate and Environment on the Complex Life Histories of Scyphozoans**

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
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3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
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6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
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A final thank you and sincere apology to my friends and family who probably learnt far more about jellyfish than they could have ever wanted to. I couldn't have done it without your help.

Ethical Approval

Neither the moon jellyfish *Aurelia aurita* or *Chrysaora hysoscella* are protected species in the area of study. Permission was obtained from the Royal Navy to access Horsea Lake, UK (50°49058.8; 1°05036.9) to collect *A. aurita* specimens. The blue blubber jellyfish *Catostylus mosaicus* is not a protected species in the area of study. All international, national, and/or institutional guidelines for the care and use of animals were followed where applicable.

Chapter 1 Introduction

1.1 Introduction

Large aggregations of jellyfish occur at global, regional, and local scales (Dawson et al., 2015). These gelatinous animals fulfil many ecological roles and are important predators and prey in a variety of ocean systems, from coasts and pelagic marine systems, to the deep ocean (Pitt et al., 2008; Roux et al., 2013). Jellyfish aid in nutrient transfer between different trophic levels, as well as carbon sequestration and transport through the water column from surface waters to the seabed through faecal pellets and jelly falls (Lebrato et al., 2012; Sweetman et al., 2014). Jellyfish also play an important role in biodiversity regulation, enhancing ecosystem biodiversity (Boero et al., 2008). Despite some problematic interactions with human industry and activity, such as clogging fishing nets and stinging swimmers, jellyfish play a large role in science, helping researchers to understand the impact of anthropogenic forcers on our ecosystems, inspiring biomechanical design and medical advances (Nawroth et al., 2012; Leone et al., 2015). Notwithstanding their ecological roles, jellyfish also contribute to the human experience, through jellyfish aquaria around the world that inspire curiosity and interest in the oceans and their inhabitants.

As a group, jellyfish share a gelatinous structure, with low carbon and high-water content (Arai, 1997). It is partly due to these features that the field of jellyfish research lags behind other areas of marine research. Traditional sampling methods by net or dredge leads to the destruction of their fragile body structure, making identification and taxonomic classification challenging (Pugh, 1989; Haddock, 2004). This has resulted in the exclusion of gelatinous zooplankton such as jellyfish from many routine long-term sampling programmes (Pugh, 1989) and to a dearth of long-term data on abundance and distribution despite the ubiquity of jellyfish across most of the global oceans (Dawson et al., 2015). Not being able to effectively collect these animals intact has led to a lack of laboratory experiments, and their functional biology and ecology was poorly understood compared to other zooplankton groups until the late 20th century (Arai, 1997). This has led to an underestimation of their importance in trophic webs and ecosystems, and to the belief that jellyfish are trophic dead ends, with few predators, that in turn has resulted in a low research prioritisation.

1.2 Scyphozoan Jellyfish

1.2.1 What is a scyphozoan jellyfish?

The Scyphozoa, or 'true jellyfish', are one of the four living classes of the Phylum Cnidaria. These organisms possess:

- Intrinsic stinging organelles called cnidae used for defence and prey capture;
- Two epithelial body layers, an epidermis and a gastrodermis separated by the mesoglea;
- A single opening, called the mouth, typically surrounded by tentacles forming a ring around the margin of the oral disk (Arai, 1997)

The Scyphozoa differ from other cnidarian classes such as the Cubozoa and Hydrozoa in lacking a velum or velarium extending into the subumbrella space, and from the Anthozoa in not having a defined pharynx (Arai, 1997). Scyphozoan species fulfil many different ecological roles and have many different shapes and sizes, contained in well-recognised orders: the Coronatae, Semaeostomeae and the Rhizomstomeae (WoRMS Editorial Board, 2021).

1.2.2 Life Cycle

Scyphozoa also distinguish themselves by having a metagenic life cycle formed of two distinct life phases: a pelagic, sexually reproducing medusa, and a benthic, asexual polyp (Figure 1.1; Lucas, 2012). There are two short-lived, intermediate larval stages: the planula larva, produced by the medusa; and the ephyra, released by the polyp (Goldstein and Steiner, 2020). This thesis is based on the study of the environmental drivers between life stages. It is therefore important to first understand the biology of each of the four life stages and the main variables influencing their health, reproduction, and survival, as detailed in the following four sections.

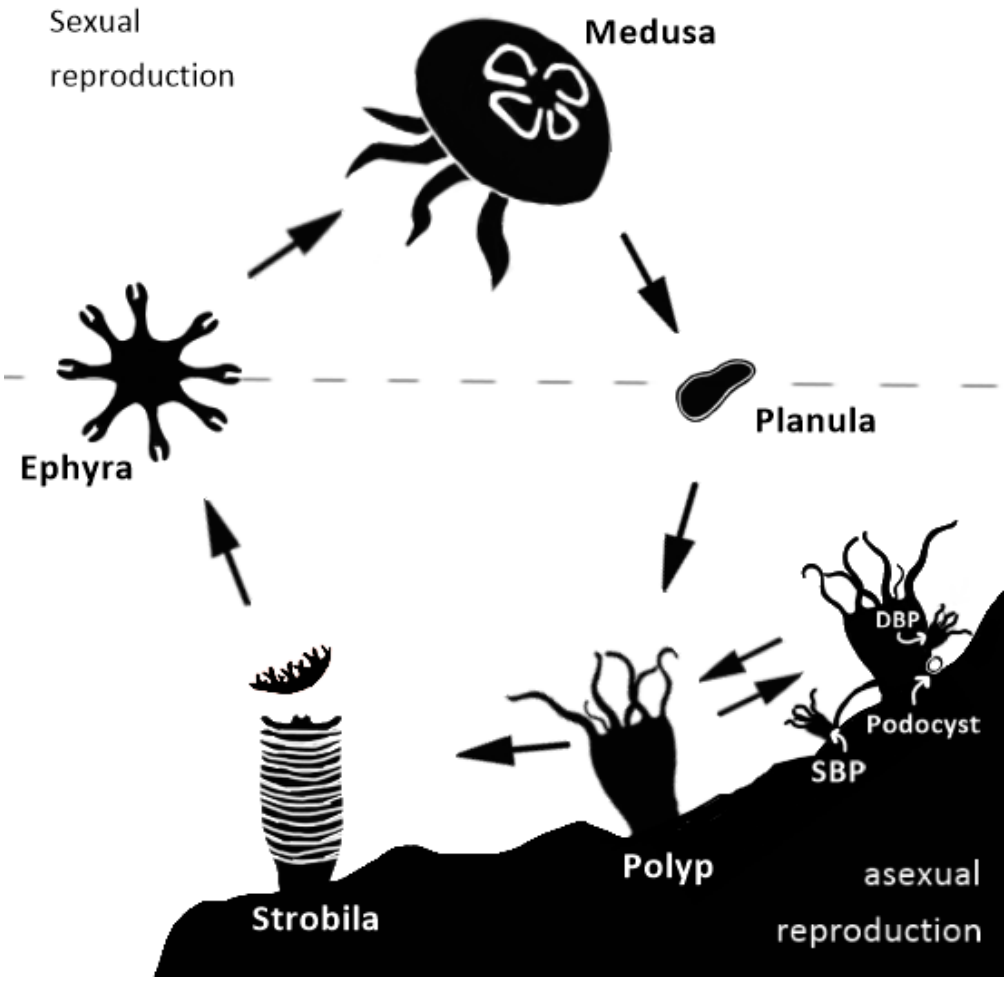

Figure 1.1. A typical scyphozoan four stage life cycle. SBP = Stolonally budded polyp; DBP = Directly budded polyp

1.2.2.1 **Medusa life stage**

Medusae represent the adult phase of the pelagic life stage of scyphozoan jellyfish. Many scyphozoans such as *Aurelia aurita* are bloom forming species, wherein populations will undergo a large seasonal increase in abundance, often with significant ecological and socio-economic consequences (Kingsford et al., 2018; Goldstein and Steiner, 2020). They are typically present for a few months to a year, and the drivers behind the large interannual variations in timing and abundance have not been fully explained by existing literature (Brotz and Pauly, 2017; Schnedler-Meyer et al., 2018a). They are likely to be the principle dispersal phase because they are reasonably long-lived (from several months to >1 year) and are planktonic (Dawson and Jacobs, 2001).

Growth and reproduction

Medusa size is determined by bell diameter, with swimming speeds increasing linearly, and wet weight increasing exponentially with bell diameter (Bailey and Batty, 1983). Growth is exponential

in the spring for temperate populations provided with enough food and increasing water temperature, with ephyrae <4 mm diameter growing to fully mature medusae by the height of summer (Möller, 1980; Hernroth and Gröndahl, 1985b). The transition between medusa and ephyra occurs when ephyrae grow larger than 10 mm diameter. Growth rates are directly influenced by food intake, and reduced somatic growth observed in the field may be due to the shunting of energy from somatic growth to reproduction (Hansson, 1997a).

Medusae are dioecious (Eckelbarger and Larson, 1988), with typically a 1:1 sex ratio, and depending on the species, eggs may be fertilised in the water column (Cargo, 1975), in the oral arms, gastrovascular cavity or the ovary (Kikinger, 1992; Arai, 1997; Schiariti et al., 2012). The onset of reproduction occurs earliest in the largest medusae, although in some populations such as those in Horsea Lake on the south coast of the UK, eventually all medusae, regardless of their size, become mature and reproduce (Brewer, 1989; Lucas and Lawes, 1998). This plasticity in the size of the reproducing animals ensures maximal reproductive output by the population (Brewer, 1989). After planula larvae release it is not unusual for medusae to cease growing and degenerate completely, independent of the concentrations of food in the surrounding water, or the size and age of the medusa (van der Veer and Oorthuyusen, 1985; Lucas, 1996; Hansson, 1997a). This deterioration and eventual death may provide transport of any remaining larvae to the benthos within the oral folds (Brewer, 1989).

Feeding and digestion

Medusae are voracious predators and can have significant impact on the abundance, biomass and community size composition of micro- and mesozooplankton at lower trophic levels (Möller, 1980; Mills, 1995; Purcell and Arai, 2001). Consequently, their influence will change depending on the time of year and stage of growth, resulting in different community structures at different times of year (Hansson, 1997b). Medusae are opportunistic predators and are known to prey upon a variety of zooplankton species (Möller, 1980; Brewer, 1989; Uye and Shimauchi, 2005), fish larvae and eggs (Möller, 1980 2001; Purcell and Arai, 2001), hydromedusae (Matsakis and Conover 1991), algae, phytoplankton (Båmstedt, 1990), and detritus (pers comm.). They use jet propulsion to create a feeding current, which entrains prey towards the mouth and tentacles (Costello and Colin, 1995). Medusae depend on the opportunistic exploitation of zooplankton aggregations such as spring plankton blooms, as the average abundance of potential prey in the water column is rarely sufficient to fuel exponential growth and many field populations are normally food limited across the rest of the year (Bailey and Batty, 1984; Båmstedt, 1990; Uye and Shimauchi, 2005). This reflects their opportunistic nature, where due to quick response times they are able to take advantage of rapidly changing conditions.

Predation and competition

Predators of medusae include the leatherback sea turtle *Dermochelys coriacea*, an obligate jellyfish predator which consumes potentially hundreds of kilograms of jellyfish a day (Heaslip et al., 2012), >100 species of fish (Arai, 1988; Ates, 1988; Ates, 2017; Mianzan et al., 2001; Purcell and Arai, 2001; Arai, 2005; Cardona et al., 2012), as well as benthic predators such as sea anemones (Jarms and Tiemann, 2004), decapods (Esser et al., 2004), spider crabs and echinoderms (Ates, 2017). Gelatinous predators of fellow jellyfish include species such as *Phacellophora camtschatica* in the Pacific (Strand and Hamner, 1988), *Cyanea capillata* in northern European waters (Båmstedt et al., 1994), *Drymonema* sp. in the Mediterranean and western Atlantic (Larson, 1987; Bayha and Dawson, 2010).

Gelatinous tissues have a higher digestion rate than other tissues and may easily be lost after collection (Arai, 2005), which may have led to multiple authors overlooking the importance of jellyfish in other organisms' diets based on sampled stomach contents (Riascos et al., 2012). As well as this, 'jelly falls', when a bloom falls to the seafloor, can provide valuable nutrients to benthic communities and opportunistic scavengers (Yamamoto et al., 2008; Sweetman et al., 2014). These play an important role in the biological pump by transporting carbon and liberating nutrients into the deep sea (Lebrato et al., 2012). Finally, medusae also compete with planktivorous finfish by eating copepods, other zooplankton, and predate on fish eggs and larvae (Möller, 1980; Båmstedt, 1990; Roux et al., 2013).

Abiotic influences

Medusae respond to a wide range of physical (Graham and Kroutil, 2001; Rakow and Graham, 2006; Fossette et al., 2015) and chemical stimuli (Albert, 2011). Distribution is consequently determined by not only polyp location, but also the abiotic and hydrographic environment into which ephyrae and subsequently medusae are released and grow. For example, variable salinity elicits a species-specific behavioural response in medusae (Albert, 2014). Medusae can detect changes in salinity and respond by swimming downwards (Albert, 2014). Large medusae swim using jet propulsion, and can maintain their position in the water column. For example, in the Saanich Inlet, British Colombia, *A. aurita* migrates directionally against the flow to maintain breeding aggregations (Hamner et al., 1994). Medusae that are injured or are not strong enough to swim against the flow are swept away from these aggregations, therefore eliminating them from the gene pool. Asides from water currents, freshwater inputs through rainfall and river export, as well as wind strength and direction also influence the distribution of jellyfish (Graham et al., 2001; Amorim et al., 2018).

In regions with large seasonal temperature fluctuations, such as in Southampton Water where seasonal temperature range from <10 to >20 °C, medusae may benefit from the increase in spring temperatures, both directly through increased growth rates, as well as indirectly through a temperature mediated increase in the standing stock of prey (Schneider and Behrends, 1994; Hansson, 1997b). Growth rates increase with rising temperature within an individual's thermal window. Conversely, degrowth, where a medusa shrinks, is correlated with a decrease in temperature, and falling temperatures are likely to have an indirect effect on medusae by limiting mesozooplankton productivity (Lucas and Lawes, 1998; Hansson, 1997b). As such, food and temperature work in combination to determine the growth rate of medusae in the spring and early summer (Hansson, 1997b). Finally, as the medusa is able to shrink and grow independently of age, quantifying an organism's age through size is not possible.

Other ecosystem services

Jellyfish, whilst being voracious predators, also provide numerous ecosystem services (Arai, 2005). For example, many species of juvenile fish have been observed associating with passive host jellyfish, for both food and protection (Riascos et al., 2012; Mianzan et al., 2013), as well as crustaceans (Hayashi and Miyake, 1968). Parasites, often used as indicators of trophic web structure, important to baseline ecological dynamics, are very common in jellyfish (Laval, 1980; Oliva et al., 2010; Riascos et al., 2012), including certain amphipods (Bowman et al., 1963; Möller, 1980; Riascos et al., 2012) and copepods (Dahl, 1961).

Issues with sampling and studying medusae

Due to their gelatinous nature, medusae are difficult to sample by net and may be injured if not handled with care (Tay and Hood, 2017). Some of the longest records of jellyfish abundance are shore-based surveys that may not be representative of the entire water column, especially in deeper water and when there are distinct hydrological features such as pycnoclines that may aggregate or restrict the vertical distribution of medusae (Graham et al., 2003; Rakow and Graham, 2006; Suzuki et al., 2016; Tay and Hood, 2017). Many surveys also use fixed stations, that may provide only a limited view of the population as changes in flow will change the observable population, causing observer bias to pick out patterns that may not actually exist (Tay and Hood, 2017). Long-term records of medusa presence and abundance are necessary to determine whether populations cycles are changing in response to environmental drivers.

1.2.2.2 Planula larva life stage

Planula larvae are free swimming lecithotrophic larvae produced by scyphozoan medusae via sexual reproduction (Gröndahl, 1989; Lucas, 2001). They are elongated, polarised stereogastrula

(Hyman, 1940; Mergner, 1971; Neumann, 1979), with a differentiated ciliated ectoderm surrounding a simple ectoderm (Widersten, 1968). This ciliation enables the larvae to move through the water column by rotating around their own axis (Calder, 1982; Pitt, 2000; Ishii and Takagi, 2003). Size, colour and settlement behaviour differ depending on the species (Calder, 1982; Lotan et al., 1992; Pitt, 2000). Although morphologically simple and lacking complex sensory organs, considerable cellular differentiation has been observed in planula larvae: neurones, sensory and gland cells, muscle extensions and nematocysts have been detected, concentrated at the anterior end of the larvae where cells bearing long cilia form an apical tuft (Hyman, 1940; Svane and Dolmer, 1995). This may function as a tactile sensory organ during substrate selection that responds to light, temperature, and gravity, as well as chemical and tactile stimuli (Burke, 1986; Svane and Dolmer, 1995). The main role of the planula larva is to act as the link between the pelagic and benthic phases of the scyphozoan life cycle as well as to select a suitable settlement site for the polyp (Brewer, 1984).

Many reports stress that the survival of the juvenile stages is critical for the success of a population. However, many of these studies focus only on the polyp stage, not on the planula larvae. Polyp location and distribution is a direct consequence of the selection of a suitable substrate by motile planula larvae (Crisp, 1974; Brewer, 1978). Consequently, mortality of the planktonic larval stage during settlement, and selection of an advantageous settlement site will greatly influence the abundance and survival of the adult benthic population (Brewer, 1976). Understanding what drives or hinders settlement will help to explain the large interannual variations in medusa abundance.

Settlement and metamorphosis

The total duration of the larval stage varies between species and locations (Schneider and Weisse, 1985; Ishii and Båmstedt, 1998), not only determined by the environment into which they are released, but also by maternal provisioning by the parent medusa. Settlement typically takes hours, but larvae can remain in the water column for over 7 days (Calder, 1982; Gröndahl, 1988b). This represents the conclusion of the pelagic life phase (Leitz, 1997). Several factors initiate settlement and metamorphosis into a polyp, including the biotic and abiotic characteristics of the substratum (Keen, 1987; Gröndahl, 1989). Settlement location differs depending on the characteristics of the substrate: some species are generalist and are able to survive within a wide range of environments, whereas others select for specific factors with which the adult is commonly associated (Brewer, 1976b; Brewer, 1976a). Planula larvae display a searching behaviour prior to settlement, wherein they inspect the site by orienting themselves vertically with their anterior end closest to the substrate for variable periods of time depending on the

quality of the substrate. If unsuitable, the planula larvae will leave the site and glide for a distance before inspecting the next site (Crisp, 1974; Brewer, 1984; Holst et al., 2007).

Much of the work on settlement, metamorphosis and the factors influencing it have examined settlement in a few species in key locations such as in the German North Sea and temperate species (Gambill et al., 2018; Holst and Jarms, 2007). *A. aurita* planula larvae have been observed to settle on a great number of living and non-living natural substrates including algae, mussel shells, barnacles, calcareous polychaete tubes, solitary ascidians, hydroids, seagrass and other naturally occurring hard substances including any space left by the removal of other fouling organisms (Lucas, 2001; Miyake et al., 2002).

Alongside the development of aquaculture and coastal structures such as breakwaters has grown the theory that artificial structures may increase the bloom potential of scyphozoans (Bulleri and Airoldi, 2005). Multiple authors have observed a preference in scyphozoan species for artificial settling surfaces (Pitt, 2000; Holst and Jarms, 2007). In laboratory and field experiments, plastic and ceramic settling plates are often densely colonised (Miyake et al., 2002). This preference would be especially important in areas of soft sediment, where polyps would not previously have been able to settle (Janßen et al., 2013). Drifting and floating objects provide possible routes for colonization and invasion of new areas, with plastic flotsam a potential habitat for polyps (Holst and Jarms, 2007; Miyake et al., 2002). However, this trend may be confounded with the fact that natural substrates are often size limited by environmental conditions and other competing organisms (Hoover and Purcell, 2009). In field and lab experiments the use of flat settling plates may artificially increase predation rates and overgrowth, as when settling in the field, polyps choose crevices and grooves that may provide refuge from predatory organisms (Colin and Kremer, 2002).

Predation

Scyphozoan planula larvae are vulnerable to predators, which partly explains their short residence time in the water column (Schneider and Weisse, 1985). Benthic predators including developed polyps may be one of the major sources of planula larvae mortality (Gröndahl, 1988b; Lucas, 2001). Inter-species predation is high, for example *A. aurita* polyps have been observed readily capturing and ingesting *C. capillata* and *C. lamarckii* planula larvae in the Gullmar Fjord (Gröndahl, 1988a).

Abiotic influences

As a non-feeding larva, following suitable provision by the parent, the environment into which a planula is released will determine survival and successful settlement to the benthos. Survival in

low salinity areas is reduced by the larva's inability to settle correctly and impaired development at low salinities (Holst and Jarms, 2007), although they are tolerant of hypoxic conditions (2-0 ml O_2/L) (Diaz and Rosenberg, 1995). Responses to other environmental variables such as temperature are not yet clearly defined. For example, lower water temperature shortens the possible period spent in the water column in some populations (Ohtsu et al., 2007; Dong and Sun, 2018). Theoretically, as increased temperature accelerates the larval metabolic rate, the survival time on body energy stores alone should be shortened at higher temperatures, therefore encouraging earlier settlement (Goldstein et al., 2017). However, increased water temperatures have also been observed to increase the larval residence time in the water column in other populations (Webster and Lucas, 2012). As such, the influence of temperature on planula larvae settlement is unclear and requires clarification.

As well as this, the hypothesis that scyphozoan larvae display gregarious settlement behaviour is contentious and there is no clearly defined answer even within one species (Keen, 1987; Burke, 1986; Gröndahl, 1989). Gregarious behaviour would confer several advantages onto polyp populations. Firstly, conspecifics are less likely to prey on any new settling larvae of the same species, although this does occasionally occur. As well as this, colonies may avoid overgrowth by other species such as barnacles or ascidians by hindering the settlement of competing species (Gröndahl, 1988b; Gröndahl, 1989). However, gregarious settling may result from the hydrographic conditions experienced by a larva (Keen and Gong, 1989).

Issues with sampling and studying planula larvae

Planula larvae are difficult to sample *in situ* due to their small size and short residence time in the water column. As well as this, when collecting gravid medusae, larvae will be lost to the water column if not handled correctly. When studying settlement in the field, the use of settling plates does not elucidate the true distribution and changes in abundance of naturally occurring populations due to competition for space on the benthos. As well as this, laboratory experiments that put multiple larvae in a single replicate treat non-independent samples as true replicates and allow confounding interaction effects within treatments, such as gregarious behaviour. Finally, potential cannibalism from faster settlers may confound the settling ratios of planula larvae.

1.2.2.3 **Polyp life stage**

Polyps, also referred to as scyphistomae, are the benthic component of scyphozoan life cycles (Lucas, 2001). Polyps contribute to the seasonal appearance of medusae by producing ephyrae via the process of strobilation under appropriate environmental conditions (Lucas et al., 2012; Fuchs

et al., 2014; Höhn et al., 2017). As such, this life stage directly governs the interannual medusa population and is key to the success or failure of a jellyfish bloom.

Growth and reproduction

A fully mature polyp has 16 tentacles supported by a strong core of cells (Arai, 1997; Riascos et al., 2013). Polyps of many species are perennial, perpetuating populations in temperate areas where the medusae disappear during the winter (Lucas, 2001; Thein et al., 2012). In the field they are typically found on the underside of objects in low light (67-140 lux) (Purcell et al., 2007).

Scyphozoan polyps reproduce through a variety of asexual modes (Adler and Jarms, 2009). The four main modes of reproduction observed in the majority of scyphozoan species include (Figure 1.2):

- **Direct, or lateral budding**: Offspring grow from the polyp body wall directly at the junction point of calyx and stalk. Budding occurs most frequently under favourable conditions (Hubot et al., 2017; Hofmann et al., 1978; Han and Uye, 2010). Direct lateral budding is the most common mode of budding observed in *A. aurita*.
- **Stolonal budding**: Offspring grow on stolons and several polyps can form along a single stolon (Adler and Jarms, 2009; Han and Uye, 2010).
- **Podocysts**: Dome shaped cysts covered in a chitinous protein sheath form beneath the basal region of the polyp or at the attachment area of stolons (Arai, 2009; Thein et al., 2012). They are whitish or light brown in colour and range from 200 to 700 µm diameter (Han and Uye, 2010). Podocysts can endure prolonged periods of poor environmental conditions such as low food supply or high temperatures (Arai, 2009). As well as producing cysts, polyps can also self-encyst, allowing them to endure adverse conditions. Podocysts contain rich organic reserves of carbohydrates, proteins and lipids that are gradually consumed (Thein et al., 2012). Excystment occurs when conditions improve (Arai, 2009).
- **Strobilation:** The polyp-to-medusa transition is divided into 3 successive phases: induction of metamorphosis, strobilation, and jellyfish morphogenesis (Fuchs et al., 2014). Segmentation starts at the apical part of the polyp and progresses downwards, with constrictions forming at equal distances (Kroiher et al., 2000). Subsequently formed ephyrae have the same orientation, leading to stacks of disk-shaped segments (Straehler-Pohl and Jarms, 2010). Polyps can produce one or multiple ephyrae per stack depending on the species, population, and environment (Lucas, 2001; Prieto et al., 2010). In the field, strobilation is a seasonal process, which occurs following an environmental cue, such as the warming or cooling or water in the spring and autumn (Kroiher et al., 2000). As such,

polyps distinguish between short-term temperature fluctuations and seasonal changes using a temperature dependent molecular timer (Fuchs et al., 2014). There are also suggestions that some populations strobilate several times a year under the right conditions, despite the high amount of mortality following strobilation (Calder, 1974; Lucas and Williams, 1994).

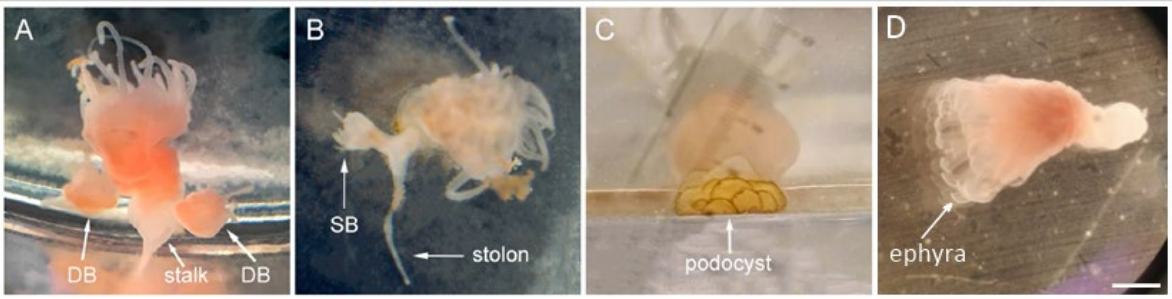

Figure 1.2. Asexual reproductive modes in *Aurelia aurita*: (A) direct buds (DB) formed directly on the polyp body; (B) stolonal buds (SB); (C) cluster of podocysts formed beneath the basal region; (D) stack of ephyrae developing on a strobilating polyp. Scale bar: 1 mm. Modified from Lu et al. (2020)

Abiotic influences

Polyps are sedentary organisms unable to escape changes in the surrounding environment. Variables that influence the growth, survival and reproduction of benthic polyps include: temperature (Willcox et al., 2007; Liu et al., 2009; Han and Uye, 2010), dissolved oxygen concentrations (Ishii et al., 2008; Ishii and Katsukoshi, 2010; Miller and Graham, 2012), competition for space (Colin and Kremer, 2002; Watanabe and Ishii, 2001), for food (Ishii and Watanabe, 2003; Purcell et al., 1999), predation (Hoover et al., 2012; Hernroth and Gröndahl, 1985a), as well as salinity and light (Miyake et al., 2002; Liu et al., 2009; Willcox et al., 2007).

Temperature is the main variable influencing growth, survival, and driving polyp asexual reproduction (Schiariti et al., 2014; Fuchs et al., 2014). Within thermal windows that constrain ectothermic polyp latitudinal distributions, temperature determines the rate at which physiological processes occur and drives the response to these changes (Fitt and Costley, 1998; Gambill and Peck, 2014). Performance is highest at the thermal optima, which reflects the seasonal temperature range of each population (Riascos et al., 2013), and temperatures beyond the thermal window of the population affect survivorship and growth (Hubot et al., 2017; Purcell et al., 2012).

Initiation of reproduction is temperature dependent and reproductive rates increase with rising temperatures up to a certain threshold (Riascos et al., 2013; Gambill and Peck, 2014; Feng et al., 2015). Varying the water temperature in the laboratory (Kroiher et al., 2000; Liu et al., 2009; Holst, 2012), or lowering it for a certain amount of time akin to the winter temperature decrease induces strobilation (Loeb, 1972; Calder, 1974), however strobilation can also be induced artificially by the introduction of iodine into the water (Spangenberg, 1967; Green and Wolfenden, 2018). Despite this research, our understanding of how temperature drives jellyfish bloom formation in changing conditions is still incomplete.

Feeding and digestion

Polyps are primarily carnivorous, feeding mostly on meso- and micro-zooplankton, although quantitative data on microzooplankton feeding is mostly limited to studies examining ephyrae and medusae (Olesen et al., 1996; Båmstedt, 1990). Polyps have also been observed to feed on phytoplankton and detritus (Båmstedt et al., 2001).

Predation and competition

Key predators of scyphozoan polyps include nudibranchs, gastropods and shrimp (Hoover et al., 2012; Hernroth and Gröndahl, 1985a). Many organisms readily consume polyps, although most avoid preying on podocysts, which may protect populations from heavy predation pressure (Arai, 2009; Hoover et al., 2012).

Variation in *A. aurita* population size likely reflects the competitive success of the benthic polyp. Competition for space involves out-competing other fouling organisms such as barnacles, ascidians and bryozoans (Toyokawa et al., 2011; Colin and Kremer, 2002). The construction of artificial substrate will consequently benefit populations only if they can reproduce successfully and expand without being outcompeted (Feng et al., 2017). More fragile polyp colonies may proliferate more successfully in harsher environments where fouling organisms cannot compete as well, such as in low oxygen environments (Condon et al., 2001).

Issues with sampling and studying polyps

As it is much easier to examine polyps cultured in the laboratory than in the field due to their small size and cryptic nature, most researchers tend to use cultured stock polyps (Willcox et al., 2007; Arai, 1997). As such, the metabolic response to environmental influencers will be influenced by acclimation time and duration of captivity, as sensitivity to variables may be shifted depending on the time of capture and acclimation period (Höhn et al., 2017). Laboratory polyps tend to have a much higher survival rate than wild polyps and longevity measurements from the lab must be

tempered with estimates gathered in the field to gain an accurate estimate (Liu et al., 2009). Multiple attempts at locating polyp colonies through the use of SCUBA diving have failed (Lucas and Williams, 1994; Han and Uye, 2010). As such, many field experiments have taken place using artificial settling plates, however this may not always reflect the true dynamics of the benthic community (Miyake et al., 2002; Watanabe and Ishii, 2001).

There is currently a lack of research on population thermal windows that limits our ability to project how warming might affect limit life cycle and bloom dynamics (Gambill and Peck, 2014). Common garden experiments are needed to test for potential physiological adaptations of polyps to local temperature regimes. As well as this, many ideas set out by studies are contradictory. This may be because of population specific responses that do not allow for extrapolation and comparison between species and populations (Feng et al., 2015).

1.2.2.4 **Ephyra life stage**

Ephyrae are the small (<10mm) juvenile medusae produced by scyphistomae through the process of strobilation (Feng et al., 2018; Lucas and Williams, 1994). Like the planula, this stage is transitory and short-lived due to the rapid initial growth (20-70% specific daily growth rate) of ephyrae supplied with appropriate food sources and suitable temperatures, during which time each individual develops into a fully functioning adult medusa (Båmstedt et al., 1999; Russell, 1970). Recruitment to the adult population is therefore dependent on the survival and correct development of the ephyra (Hernroth and Gröndahl, 1985b). The discontinuous ephyral lappet-cleft morphology is a conserved trait among different scyphozoan species, however it is followed by highly diverse adult morphologies (Russell, 1970). Due to a paucity of experiments examining ephyrae, data from research on other life stages have been used as a comparison in a number of ephyral studies (Faimali et al., 2014). In recent years, multiple biomechanical and toxicological studies have been carried out on ephyrae, and in 1994 *A. aurita* ephyrae were the subject of a microgravity experiment carried out in space (Dong et al., 2017; Spangenberg et al., 1994; Spangenberg et al., 1989).

Growth

Ephyrae are a transitory stage during which each individual develops from a newly released ephyra with lappets and clefts (2-4mm diameter) into a potentially huge adult with an unbroken bell (>2m in some Arctic varieties of *Cyanea capillata*) (Higgins et al., 2008). The change in morphology and behaviour associated with this growth coincides with a shift in fluid regimes from a viscous to an inertia dominated flow (Feitl et al., 2009; Nawroth et al., 2010). This causes alterations in prey transport and capture location distributions (Higgins et al., 2008). Prey

selection during development also changes as prey items that were not previously susceptible to entrainment are now caught in the stronger flows of the larger medusa as bell diameter increases (Costello and Colin, 1994). The impact of scyphozoan predators on communities will therefore change depending on the stage and size of the ephyrae and medusae predating on the community (Higgins et al., 2008).

Maximum ephyrae abundance typically occurs soon after release in late winter or early spring, but in most temperate locations they are recruited to the plankton in early spring (Lucas et al., 1997; Hay et al., 1990). In areas with constant strobilation ephyrae are produced year-round, resulting in semi-continuous recruitment to the medusae (Papathanassiou et al., 1987; Lucas, 2001). In locations where the majority of ephyrae are produced in the autumn, the autumnal generation often undergoes a winter diapause, actively migrating after release to deeper water layers (Möller, 1980; Hernroth and Gröndahl, 1983).

Predation and competition

Ephyrae feed on a high diversity of prey including small zooplankton (Båmstedt et al., 2001), cirripedes, *Artemia* nauplii as well as tintinnids and dinophyceae for younger ephyrae (Zoccarato et al., 2016). Growth rates vary between species, food sources and time exposed to prey items (Olesen et al., 1994; Olesen et al., 1996). Some food sources do not contain the correct nutritional requirements for complete development, but many of these lead to at least an initial increase in average diameter (Båmstedt et al., 2001). Phytoplankton may play an important role in sustaining newly developed ephyrae if release coincides with a phytoplankton bloom, however in most cases ephyrae must switch to a more nutritious diet if development is to continue (Båmstedt, 1990).

Interannual variation in ephyral abundance may be controlled by predation, although specific predators have not been confirmed, and there is no apparent cannibalism (Möller, 1980). As the occurrence of ephyrae in the water column is dependent on polyp strobilation, predation on the benthic polyp stage has a large impact on the pelagic population (Möller, 1980; Hernroth and Gröndahl, 1985b).

Abiotic Influences

Temperature affects ephyral growth efficiency: at low temperatures (i.e. in winter and early spring), both growth rate and efficiency are low, especially for ephyrae undergoing diapause. Growth accelerates in late spring when the water temperature increases (Hernroth and Gröndahl, 1983). Ingestion rate increases with both temperature and age (Båmstedt et al., 1999). This may be partly due to faster physiological processes at higher temperatures: digestion and pulsation rate are likely to be accelerated at elevated temperatures (Båmstedt et al., 1999). *A. aurita*

ephyrae released in marinas and coastal areas, where polyps are likely to be located due to an abundance of hard surfaces for colonisation, are likely to be subject to fluctuating salinity and temperature (Dillon, 1977), yet few studies have investigated the effects of salinity on growth and feeding in ephyrae.

Ecotoxicology studies

In recent years, interest in *A. aurita* ephyra as a model organism for ecotoxicological studies has increased due to suggestions that it is more sensitive to changes in concentrations of toxins than other model organisms and adult medusae (Faimali et al., 2014). As well as this ephyrae are easily sourced and maintained in the laboratory (Costa et al., 2015). Some tested compounds include tea saponin (Dong et al., 2017), cadmium nitrate ($CsNO_3$) and sodium dodecyl sulphate (SDS) (Faimali et al., 2014), eserine (ES) and chlorpyrifos (CPF) (Costa et al., 2015), as well as high concentrations of the toxic strains of the phytoplankton *Alexandrium catenella* (Huang et al., 2014).

Issues with sampling and studying ephyrae

In populations with semi-continuous recruitment, such as in Southampton Water, estimating growth rates can be an issue, with potential for underestimation as new individuals are recruited to the population (Lucas and Williams, 1994). As well as this, field studies measuring ephyral growth based on diameter measurements will be of low precision, since conversions from measured diameter to body mass will be imprecise unless the study follows the same group of individuals over time. In the laboratory container volume is important to consider when calculating clearance rates as the clearing rates of planktonic species rises with increasing container volume. Reduced clearance in small containers have been demonstrated for gelatinous zooplankton (Purcell and Nemazie, 1992) and the searching behaviour of jellyfish predators may be impaired in small containers due to more frequent contact with the walls that leads to increased bell pulses (Reeve, 1980).

1.2.3 Study species

This thesis is based on the analysis of data collected both in the laboratory and *in situ*. It is necessary to understand the individual-level biology and ecology of each species to be able to effectively interpret data at a bigger scale. In the following sections, a brief overview of each study species is provided.

1.2.3.1 *Aurelia aurita*

Aurelia aurita (Linnaeus, 1758), also known as the common moon jellyfish, inhabits nearshore waters around the globe between 50 °N and 55 °S (Dawson and Jacobs, 2001). It is a useful research species within the Semeaostomeae due to its wide distribution and typical scyphozoan metagenic life. Multiple sister species have been uncovered (Dawson and Martin, 2001). The medusa life stage can be recognised by the presence of four conspicuous horseshoe shaped gonads, and short tentacles lining the bell margin (Russell, 1970). *A. aurita* medusae release fertilised eggs into the oral arms over an extended period of time, consequently the state of ripeness of each individual is unknown as both fertilised eggs and planula larvae are present at the same time in the oral arms (Lucas, 1996).

1.2.3.2 *Chrysaora hysoscella*

Chrysaora hysoscella (Linnaeus, 1767), or the compass jellyfish is most commonly found in coastal areas of the northeast Atlantic Ocean, particularly in the Celtic, Irish, and North Seas (Houghton et al., 2007), as well as in the Mediterranean Sea and coastal regions of South Africa (Doyle et al., 2006; Mariottini and Pane, 2010; Hays et al., 2008). Three *Ch. hysoscella* polyps were discovered on bivalve shells sampled from the Dogger Bank, following DNA testing of samples (van Walraven et al., 2020), however no polyp colonies have been located in UK coastal waters despite medusae being frequently observed in UK waters from May to September (Russell, 1970). Most medusae have a clear exumbrella with sixteen brown V-shaped markings running from a dark apical circle or spot to the margin of the bell, as well as brown marginal lappets however there is a large amount of variation in colour patterns (Russell, 1970). *Ch. hysoscella* medusae have twenty-four marginal tentacles in groups of three alternating with eight marginal sense organs (Russell, 1970).

1.2.3.3 *Catostylus mosaicus*

Catostylus mosaicus (Quoy & Gaimard, 1824), or the blue blubber jellyfish, is a large rhizostome jellyfish endemic to eastern Australia, from Port Phillip (Melbourne) in Victoria, to the Torres Strait (Kramp, 1965). At least two subspecies in southeastern Australia have been recognised (Dawson, 2005b). Located largely in estuarine waters and bays around the North and East coast of Australia, *C. mosaicus* forms dense aggregations in surface waters (Pitt and Kingsford, 2000). The species has different colour morphs, from blue, to yellow or white, to dark brown, with colour inconsistent between geographical locations. Medusae commonly grow to from 250 - 300 mm bell diameter, and have a life span of approximately 10 months (Pitt and Kingsford, 2000). A developmental fishery was initiated in New South Wales in 1997, however this was later discontinued (Kingsford et al., 2000). Both mono and polydisk strobilation has been observed, and

C. mosaicus displays the typical alternation of medusoid and polypoid generations observed in other Rhizostome species (Pitt, 2000).

1.3 Jellyfish and Humans

Negative perceptions of jellyfish in the media and within the general public has resulted in multiple misconceptions and a lack of understanding of the ecosystem services that jellyfish provide (Pitt et al., 2018; Sanz-Martín et al., 2016). Interruptions to fishing and aquaculture industries, clogging power plants and the effects of stings on human health and tourist activities are a few of the reported socioeconomic impacts of jellyfish, and especially jellyfish blooms, on human activities (Kingsford et al., 2018; Bayha and Graham, 2014). However, excluding the many services jellyfish naturally provide to other animals in their ecosystems, as noted in the medusa section, jellyfish generate tourist revenue and provide aesthetic value in aquariums (Graham et al., 2014). Medusae are used for a number of different purposes, including for eating, medicine (Leone et al., 2015), partial feedstock for animals (Hseih et al., 2001), feed in aquaculture (Wakabayashi et al., 2012), bait (*Nemopilema nomurai* used for sea bream fishing; Omori, 1981), cosmetics and pharmaceuticals (Addad et al., 2011), fertilizer (Hussein et al., 2015), and in absorbent and biodegradable material that could be used in products such as diapers and paper towels (Shamah, 2014). Medusa biomechanics have also influenced the field of design engineering (Gemmell et al., 2013; Nawroth et al., 2012).

Global landings of jellyfish by medusae fisheries recently exceeded 1 million tons (Brotz and Pauly, 2017; Brotz et al., 2017). The FAO reports edible species as '*Rhopilema* spp.' although as many as 35 species of jellyfish have been eaten by humans, with the majority from the Rhizostomeae (FAO, 2007). Medusae are often caught using a dip-net that minimizes bycatch, habitat damage, and conflict with other commercial fisheries operating in the area, whilst promoting catch quality (Brotz et al., 2017). However, like all fisheries, medusa fisheries are at risk from over-fishing, and whilst many species are viewed as a pest, most jellyfish provide valuable ecosystem services (Brotz and Pauly, 2017). Unlike many other commercial species such as fish, the jellyfish lifecycle, more specifically the perennial benthic polyp, may provide a buffer against overfishing, however populations remain vulnerable as demonstrated by the decline in *Rhopilema esculentum* catches in Chinese waters (Dong et al., 2014).

1.4 Future Climate change

There is unequivocal evidence that the ocean system is changing, strongly attributed to anthropogenic forcing (IPCC, 2019). In the upper layers of the ocean (0-700 m), heat uptake has

more than doubled from 3.22 ± 1.61 ZJ yr^{-1} between 1969-1993, to 6.28 ± 0.48 ZJ yr^{-1} between 1993 to 2017 (Belkin, 2009). Since 1970 the oceans have taken up more than 90% of the excess heat in the climate system. Global ocean warming is not the only consequence of anthropogenic forcing. Marine heatwaves have increased in both intensity and frequency, ocean pH has declined by 0.017–0.027 pH units per decade since the late 1980s. Along with an unprecedented decline in the global cryosphere, global mean sea level has risen by roughly 0.16m from 1902 to 2015, with the majority of rise across the period 2006-2015. This rise is not globally uniform and can vary ±80%. As well as this, precipitation, winds, and extreme sea level events have increased in magnitude and frequency (IPCC, 2019).

These changes have had a dramatic effect on marine species, forcing range shifts and changes to seasonal activities, resulting in shifts in species composition, abundance and biomass that have cascading effects on ecosystem structure and functioning (Klein et al., 2017; Shanks et al., 2019; Edwards and Richardson, 2004). This has also impacted ecosystem services with both positive and negative impacts for human activities (Lynam et al., 2011; Poloczanska et al., 2013).

1.5 Are jellyfish blooms increasing?

Problematic jellyfish blooms occur in many locations around the world, and a trend in recent literature suggests that jellyfish blooms may in fact be increasing in size and frequency in concert with anthropogenically-induced climate warming. This may be in part due to their substantial coverage in media and scientific literature garnering negative attention, with jellyfish blooms becoming synonymous with symptoms of degrading natural systems (Jackson et al., 2001; Roux et al., 2013). This bias could reflect a distortion of the scientific discourse surrounding these ideas (Pitt et al., 2018; Sanz-Martín et al., 2016), in part due to the pervasive idea that “jellyfish”, or indeed “gelatinous zooplankton”, respond in similar ways across the taxa. In fact, gelatinous zooplankton are an ecologically and morphologically distinct group, despite surface level similarities (Dawson and Jacobs, 2001; Pugh, 1989). As of yet, the claim that jellyfish blooms are increasing is unsubstantiated by scientific data, due to a dearth of long-term datasets for jellyfish populations that when available, are confined to only a few species and locations (Pitt et al., 2018).

Ectothermic animals such as jellyfish have specific temperature limits, which determine their latitudinal distribution, response to warming or cooling events and sensitivity to climate change (Ishii and Tanaka, 2005; Gambill and Peck, 2014). How each life stage responds to climate change differs due to the varied environments they experience, and it is important to identify which life stages may represent thermal bottlenecks in the scyphozoan life cycle (Dahlke et al., 2020).

Comparing results from previous studies is complex due to the use of varied experimental designs, with differing experiment lengths (6-60 days long), observation frequencies (continuous or begin/end), proxies for size and growth (mouth disc, calyx, tentacles), as well as the use of different species from varying locations. Many indicators of climate change are frequently studied in isolation of each other, and the field could benefit from experiments that take a more realistic, more comprehensive, if more complicated approach to experiment design to provide solid evidence as to whether jellyfish blooms are increasing due to anthropogenically-induced climate change.

1.6 An increasingly local response to forcing factors

Genetics drive species, population and local level adaptation resulting in animals reacting differently to environmental conditions (Höhn et al., 2017). Over longer time scales these responses may result in isolationism and eventually speciation (Dawson and Jacobs, 2001; Dawson, 2005a). For example, population-specific thermal tolerance windows are as narrow as possible to reduce metabolic costs (Pörtner et al., 2007). As such, thermal windows can change over time as well as between life stages and can adapt to shifting environmental conditions: over the short-term polyps from higher latitudes are able to maintain their respiration rates at temperatures exceeding their thermal range (Höhn et al., 2017). These thermal optima are increasingly being found to differ between populations and life stages; for example, polyps survive at low winter temperatures where medusae disappear (Calder, 1982; Höhn et al., 2017). *Aurelia* spp., often referred to as a cosmopolitan generalist, may show wide intra and inter-specific plasticity in terms of eco-physiological acclimation and evolutionary adaptive responses to environmental conditions, which reflects its success worldwide (Scorrano et al., 2016; Hubot et al., 2017). However increasingly, responses of one population cannot be applied generally across a whole taxon or even species.

1.7 Aims and Objectives

Jellyfish population cycles and bloom events occur at global, regional, and local scales. Understanding what causes these cycles now and in the future is a major question in jellyfish bloom research, because of the potential impacts on ecosystem function and services. Most bloom forming scyphozoan jellyfish have complex life histories involving a long-lived asexually reproducing benthic polyp and asexually reproducing pelagic medusae. Environmental and climate factors affect each life stage, but we do not fully understand:

i) How important each life stage is to the overall population cycle;

ii) Which life stage is the most affected by varying environmental conditions; and

iii) How demographic differences in survival, growth and fecundity translate into visible jellyfish blooms.

The aim of this PhD is to undertake a comprehensive laboratory and field-based study of the physicochemical conditions that control survival, fecundity and phase transition of the different life stages of scyphozoan jellyfish, particularly focusing in the understudied bentho-pelagic coupling from the polyp to the juvenile ephyra life stages. This is a novel approach, with very few studies investigating the whole life cycle or focusing on life stage transition. By carrying out this research, we aim to highlight potential bottlenecks in the jellyfish life cycle.

Chapter 2 Extreme changes in salinity drive population dynamics of *Catostylus mosaicus* medusae in a modified estuary

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2.1 Abstract

Modifications to estuaries through the construction of barrages alter the natural dynamics of inhabitant species by controlling freshwater inputs into those systems. To understand the effects of modified freshwater flows on a native scyphozoan jellyfish, *Catostylus mosaicus*, and to identify the environmental drivers of medusa occurrence, we analysed a 20-year observational dataset composed of 11 environmental variables and medusa presence/absence from 15 sampling stations located below the Fitzroy Barrage, in the Fitzroy River, Queensland. Major decreases in salinity (minimum salinity 0) occurred approximately 16 times during the 20-year period and medusae disappeared from the estuary following every major freshwater flow event. Salinity was identified as the most influential variable contributing to variation in the number of upper estuary sites reporting jellyfish. We then ran two laboratory experiments to test the following hypotheses: (i) prolonged decreases in salinity impair survival, pulsation, and respiration rates of *C. mosaicus* medusae; and (ii) transient decreases temporarily impair pulsation and respiration but medusae recover when salinity returns to normal levels. Medusae were unable to survive extended periods at extreme low salinities, such that they would experience when a barrage opens fully, but had significantly higher survival and recovery rates following smaller, transient changes to salinity that might occur following a moderate rainfall event. This demonstrates for the first time that modification of freshwater flow by a barrage regulates the population dynamics

of an estuarine jellyfish, and highlights the need for robust, long term datasets, and to firmly embed experimental approaches in realistic ecological contexts.

2.2 Introduction

Estuaries are highly dynamic environments that are often subject to rapid changes in environmental conditions associated with varying freshwater flows (Morais, 2008; Gillanders and Kingsford, 2002). Modifications to natural flow, through the construction of dams and barrages used to store freshwater and mitigate flooding, alter the natural dynamics within estuaries, with potentially severe consequences for estuarine species (Xian et al., 2005; Montagna et al., 2012; Amorim et al., 2018). Whilst estuarine species can generally tolerate reasonable salinity fluctuations (Lee et al., 2017; Heim-Ballew and Olsen, 2019), extreme reduced salinity is often detrimental to animals (Holst and Jarms, 2010; Amorim et al., 2018). Determining how species respond to modifications in their environment will enable stakeholders to better manage estuaries and waterways (Gillanders and Kingsford, 2002; Amorim et al., 2018; Bunn et al., 2010).

Jellyfish are important components of many coastal and estuarine systems and frequently form spectacular blooms that affect ecosystem structure and marine food webs (Goldstein and Steiner, 2017; Schnedler-Meyer et al., 2018a; Goldstein and Steiner, 2020). Scyphozoan jellyfish life cycles comprise a pelagic sexual medusa and a benthic asexual polyp (Lucas et al., 2012). Their ability to take rapid advantage of favourable conditions, coupled with the medusa lifespan of a few months (Pitt, 2000), leads to transient population booms, termed ‘jellyfish blooms’ (Schiariti et al., 2014). Interactions between polyp colonies and their environment lead to variations in the timing and appearance of these blooms, with some evidence that anthropogenic disturbances may modify natural dynamics (Purcell, 2012; Pitt et al., 2018). In coastal areas, blooms often interfere with human enterprise and recreation (Purcell et al., 2007; Kingsford et al., 2018), but jellyfish also provide valuable resources to pharmaceutical and scientific industries (Leone et al., 2015; Brotz et al., 2017). Determining how changes to environmental flows affect estuarine jellyfish populations will help stakeholders understand their population dynamics and provide insights into managing potentially problematic species (Jones and Moss, 2011).

Although many species of gelatinous zooplankton inhabit estuaries that are subject to varying salinity (Brewer, 1989; Wang et al., 2016; Baumsteiger et al., 2018), only two studies have examined how varying freshwater flows affect jellyfish medusae (Xian et al., 2005; Amorim et al., 2018). Most research examining scyphozoan jellyfish populations in estuaries has focused on settlement of planula larvae and polyp reproduction, and on how salinity changes affects medusa production (Conley and Uye, 2015; Takao and Uye, 2018). Both the planula and polyp stages have

limited capacity to move away from areas of reduced salinity (Webster and Lucas, 2012). Thus, low salinity may hinder bloom formation because it inhibits larval settlement and increases morphological deformities in polyps, which impairs their ability to feed (Cargo and King, 1990; Conley and Uye, 2015; Widmer et al., 2016). As osmoconformers, medusae have limited ability to respond to changes in salinity, and if subjected to rapid changes the animal will shrink or swell (Albert, 2014). Some species of pelagic medusae actively avoid low salinity in surface waters by swimming below the halocline (Albert, 2014). Consequently medusae are generally observed in higher salinity areas of estuaries, although further details on how they respond to changes in salinity are still lacking (Albert, 2014; Heim-Ballew and Olsen, 2019; Amorim et al., 2018).

Catostylus mosaicus, commonly referred to as the Blue Blubber jellyfish, is a rhizostome medusa native to the Australian east coast and the Central Indo Pacific (Pitt and Kingsford, 2003b; Purcell et al., 2013). The medusae are large, frequently growing to bell sizes of 250-300mm (Pitt, 2000) and have been harvested in small quantities in Australia and Asia (Kingsford et al., 2000; Omori, 1981). Despite a growing commercial interest in this species and the medusae being abundant in many eastern Australian coastal lagoons and estuaries (Dawson, 2005a; Dawson et al., 2015), little is known about the drivers behind the spatial and temporal patterns of occurrence of *C. mosaicus* medusae. Sightings outside of estuaries and coastal embayments are rare (Kingsford et al., 2000) and although anecdotal observations indicate that medusae may endure short-term reductions in salinity, such that may occur following rainfall, they are unlikely to survive reduced salinity for multiple weeks to months (Pitt and Kingsford, 2000). Severe modifications of freshwater flows, therefore, could result in significant changes to medusa population dynamics (Amorim et al., 2018).

We aimed to understand how the modification of an estuary (in this case a barrage used for flood mitigation) affects the population dynamics of *C. mosaicus* medusae through two different approaches. Firstly, we used a 20-year data set to identify how the occurrence of medusae related to environmental parameters. Secondly, we conducted two laboratory experiments to investigate the effect of salinity changes on medusae health and survival, by simulating: (i) severe and prolonged reductions in salinity, mimicking a barrage opening; and (ii) transient reductions in salinity, simulating a moderate rainfall event. The following hypotheses were tested: (i) prolonged decreases in salinity impair survival, pulsation, and respiration rates of *C. mosaicus* medusae; and (ii) transient decreases temporarily impair pulsation and respiration but medusae recover when salinity returns to normal levels.

2.3 Methods

2.3.1 Study Site

The Fitzroy River estuary (S 23°48.643', E 150°65.309') is a shallow, macrotidal estuary in sub-tropical Queensland, Australia (Dobbie et al., 2003). The Fitzroy Barrage, 53km upstream from the river mouth, separates fresh water upstream from the tidal salt water downstream and is used to mitigate flooding. Flow and mixing in the estuary and adjacent bay are most strongly controlled by freshwater flows with two hydrological states observed below the barrage: low and high (>1000 ML day^{-1}) flow (Dobbie et al., 2003; Douglas et al., 2005). Rainfall patterns largely determine freshwater discharge into the estuary (Margvelashvili et al., 2003; Radke et al., 2010), with heavy rain in the austral summer from December to February (125.1 mm rainfall/month and reduced rainfall in the winter from June to August (32.2 mm rainfall/month) recorded at Rockhampton Aero (BOM, 2021b). In a high flow state salinity can be reduced to zero 30 km below the barrage, while turbidity increases from <50 to >250 NTU (Dobbie et al., 2003). At the barrage, the river is ~200m wide with minor flood events declared at a river height of 7 m, major at ~8.5 m, however, during low flow periods the river height is typically much lower, sometimes <1 m (BOM, 2021a). River depth increases as the estuary widens, and river width increases with distance downstream, to almost 9 km wide at the mouth (Currie, 2005). Marine conditions return over a period of 4-6 months following cessation of freshwater flow, consistent with the exchange time for the estuary water of 100 days. The estuary depth varies from 3 to 7 m at mid tide with the tides varying from 0.3 to 4 m near the barrage and at the mouth, respectively. Tides are mixed, dominant semidiurnal and follow a two-weekly cycle of spring tides and neap tides. When the barrage is closed, flows are generally small (median 7 m^3s^{-1}) and tidal currents cause fine sediments (<100 µm) to be pumped upstream (Margvelashvili et al., 2003).

2.3.2 Effects of freshwater discharge on the occurrence of medusae

Each month from April 1999 to February 2019, 11 environmental variables (chlorophyll-a; phaeopigments; total nitrogen (TN), nitrogen ammonia (NH3), oxygen, pH, total phosphorous, salinity, specific conductance at 25 °C, turbidity, Secchi disk depth) were monitored along the Fitzroy Estuary at 15 sites below the barrage by the Queensland Department of Environment and Science (Appendix 1). No observations were taken in 2001, and only one observation was recorded in 2002, resulting in a total of 538 observations across the 15 sites. Sampling sites were grouped into three regions; Upper Estuary (5 sites), the first of which was located immediately below the barrage, with the others extending 9 km downstream; Mid Estuary (4 sites), extending from 15 to 32 km from the barrage; and Lower Estuary (6 sites) 41 km to 59 km from the barrage

(Figure 2.1). The number of sites at which *C. mosaicus* was observed by eye at the surface of the water each month was recorded within each of the three regions.

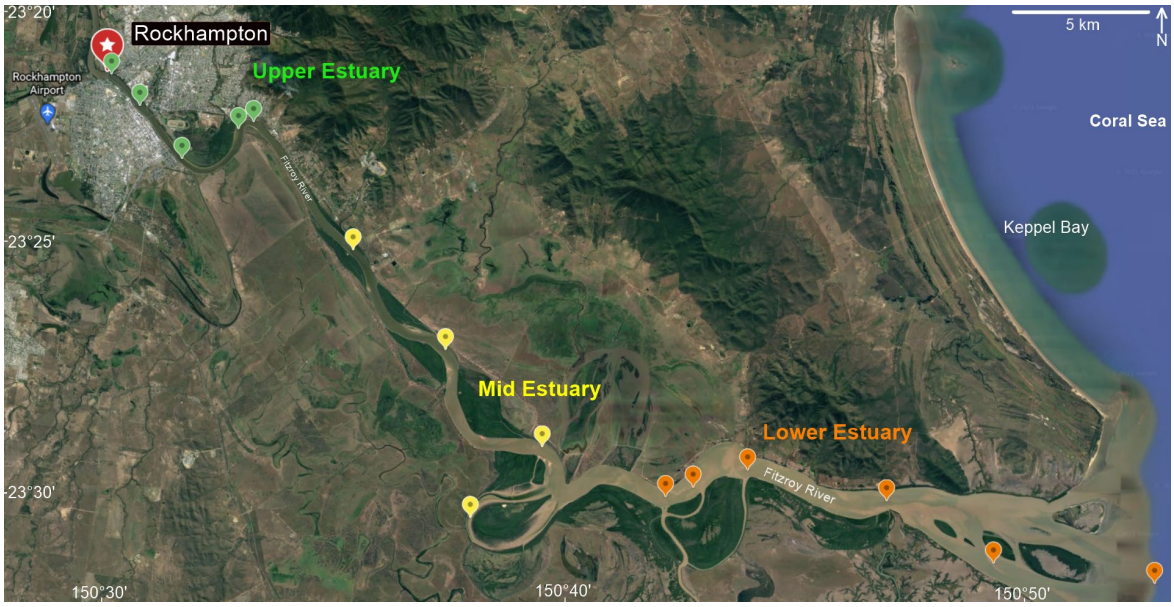

Figure 2.1. Map of the Fitzroy Basin sampling points divided by area (Upper, Mid, Lower estuary). Red star indicates the location of the Fitzroy Barrage (23.3593° S, 150.4994° E). Base map sourced from Google Earth

2.3.3 Data Analysis

The relationship between the occurrence of *C. mosaicus* and environmental variables was tested within each region using partial least squares regression models (PLSR, Appendix 1). PLSR combines features from principal component analysis (PCA) and multiple regression. It reduces a large set of predictor variables to a smaller set of uncorrelated latent variables (equivalent to principal components in PCA) and is well suited for dealing with multicollinearity in datasets, allowing for correlated explanatory variables to be included in the analysis (Rosipal and Krämer, 2005; Carrascal et al., 2009).

All variables were standardised before PLSR models were fitted using the orthogonal scores algorithm (NIPALS algorithm). Cross validation of the model using leave-one-out segments determined the appropriate number of latent variables, as well as a cross-validation error Root Mean Square Error (RMSE) plot. Variable Importance in Projection (VIP) coefficients were extracted from the relevant latent variables to determine which of the initial variables significantly influenced the occurrence of medusae within the model. VIP scores were calculated as the weighted sum of the squared correlations between the latent variables and the original variable. The weights were calculated separately for each latent variable and were weighted proportionally to the reduction in the sums of squares for each latent variable. Despite PLSR's

ability to take multicollinearity between variables into account, the VIP score cut-off value was selected as 1.5 for very important variables, and 1 for relevant variables. These levels were selected due to very high multicollinearity between numerous variables as well as the lack of consensus within the modelling community as to which cut-off value to use (Chong and Jun, 2005; Chi-Hyuck et al., 2009). Finally, regression coefficients were extracted for each model, to inform on how important variables influenced the presence of medusae in each region. Analyses were done in R v3.6.3.

2.3.4 **Experimental simulations: Prolonged vs Transient freshwater event**

Medusae were collected on 8th January 2020 from Deception Bay, Queensland (S 27°10.800', E 153°03.292'). Surface water temperature was 29.7 °C and salinity was 35. Thirty-two medusae of varying sizes were collected and transferred in buckets to the Griffith Sea Jellies Research Laboratory.

Medusae were maintained in a 12:12 hr light:dark regime and were each fed twice daily with 150 mL one-day old *Artemia* nauplii mixed at a concentration of 1600/mL with 0.1 g Polyplab Reef-roids, a dried crushed zooplankton mix. Ten to fifteen percent of water was replaced daily with water of the same temperature and salinity. Seawater was sourced from a depth of ~1 m from the Gold Coast Broadwater on a flooding tide and filtered through a 10 µm felt filter and passed over a protein skimmer. Reduced salinity water was created by adding fresh tap water (treated with sodium thiosulfate to neutralise chlorine) to seawater until the desired salinity was achieved.

2.3.4.1 **Prolonged freshwater event**

Individual medusae of similar sizes (wet weight; 511 g ± 108 SE) were placed into sixteen 87L (+65 L sump) kreisels at ambient salinity (34.5±1). The experiment consisted of four salinity treatments; a control maintained at 34.5 and three experimental salinities of 30, 20, and 10 (± 0.5). Four kreisels were randomly allocated to each treatment. Kreisels were randomly interspersed across a temperature-controlled room maintained at 24±1 °C. Salinity was decreased over 6 hours until the target salinity for each treatment was achieved. Medusae were maintained at experimental salinities for 12 days, or until they recorded no pulsating across three consecutive days and began to disintegrate.

2.3.4.2 **Transient freshwater event**

Sixteen medusae of similar sizes (241 g ± 81g) were placed in individual 87 L kreisels (+65 L sump) at ambient salinity (33±1). Due to increased rainfall at the time of the experiment, average ambient salinity was 1.5±1 lower than for the previous experiment. To simulate a rainfall event

the salinity of each kreisel was lowered over 6 hours to one of three different salinities (20, 15, and 10) or maintained as a control at ambient salinity. Salinity remained at experimental levels for 48 hours, before it was increased over 4 days (Klein et al., 2016) to ambient salinity. All medusae remained at 33±1 for the remaining 12 days of the experiment.

2.3.4.3 **Response variables**

In both experiments, survival and pulsation rates were recorded daily prior to feeding. Pulsation rates were measured as the number of complete pulsations per minute, averaged across three 1-minute intervals recorded 15 seconds apart. Respiration rates were measured prior to feeding on Day 0, 2, 7 and 12 for experiment 1, and Day 0, 3, 8, 13, and 18 for experiment 2. Respiration was measured using airtight 23 L Perspex chambers, each fitted with an oxygen sensor spot (OXSP5; Pyroscience). Sensor spots were calibrated using a 2-point (100% and 0% oxygen saturation) calibration. O_2 concentrations were measured via a compatible FireSting O2 (FSO2-4) oxygen and temperature meter fitted with an oxygen fiberoptic sensor (3 mm tip diameter). Two medusae were incubated individually for 2 hours alongside a blank filled with seawater of the same salinity. Oxygen concentrations (mg L^{-1}) were measured every 30 minutes. Oxygen consumption (µg O_2 hr^{-1} g^{-1}) was calculated using the slope of the five measurements. For logistical reasons, the 16 medusae were measured over 2 days, and each medusa was randomly assigned Group A or Group B at the start of the experiment to determine on which day they were measured. Within each group the order in which they were measured was random.

2.3.5 **Analysis of experimental data**

Assumptions of normality and homoscedasticity were tested using residuals and Q-Q plots. Survival of medusae differed among treatments creating unbalanced data sets. Subsets of data including only treatments where all 4 medusae were alive were analysed to prevent an unbalanced analysis. Where significant differences occurred, post hoc Tukey HSD tests identified which means differed.

Prolonged freshwater event - Separate ANOVAs were used to compare pulsation rates among all treatments on Day 0, 2 and 3. A repeated measures ANOVA compared pulsation rates between the control and 30 treatment across all 12 days of the experiment. Separate ANOVAs were done to compare respiration rates among all treatments on Day 0 and 2; and between the control and 30 treatments on Day 7 and 12.

Transient freshwater event - Pulsation rates were analysed using separate ANOVAs on Day 0, 2, and 8. A repeated measures ANOVA compared the control and 20 pulsation rates across 18 days.

Respiration rates were analysed using ANOVA. Comparisons were made among all four treatments on Day 0 and 3; among the control, 20, and 15 treatments on Day 8; and between the control and 20 treatments on Day 13 and 18.

2.4 Results

2.4.1 Effects of freshwater discharge on occurrence of medusae

Salinity was identified as the main variable influencing medusae occurrence in the Upper estuary. Major decreases in salinity occurred approximately 16 times over the 20-year period in the Upper estuary and medusae disappeared from the Upper estuary following every major flow event (Fig. 2.2). After cross validating the PLSR model, two latent variables (LVs) captured 40.54% of the variation in the predictors (Table 2.1; LV1: 31.18%; LV2: 9.36%), and 47.16% of the variation in the outcome variable (LV1: 40.63%; LV2: 6.53%). Additional latent variables provided minimal improvement (14 LVs = 48.24% of variation in the outcome variable). 'Salinity' and 'Specific Conductance at 25°C' were identified as the most influential variables contributing to variation in the number of Upper Estuary sites reporting jellyfish, both with VIP scores >1.5. Other relevant variables in order of importance included 'Secchi Disk Depth', 'O_2' and 'Month'. Any variables that scored below 1 were discounted as being non-important variables. Notably, despite high correlation with 'Secchi Disk Depth', 'Turbidity' was not an important factor contributing to jellyfish occurrence in either of the latent variables (VIP score <1).

Within the Upper Estuary, more sites reported sightings of medusae at higher salinities (Figure 2.2). Jellyfish were only observed at multiple sites when salinity was >10, and jellyfish were reported occurring in salinity <10 only three times across the whole dataset. With every salinity increase of 5, jellyfish were reported from approximately one additional site in the Upper Estuary. Visibility in the Upper Estuary was often <1m, with only 5 reports of Secchi disk depth >1m. The average Secchi disk depth was ~0.4m, and jellyfish were observed more frequently when Secchi disk depth was deeper, i.e. the water was clearer. Most freshwater inputs occurred in the first three months of the year, and 'Month' and 'Salinity' were correlated ($r=0.52$, $p<0.001$). Both 'O_2' and 'Month' were barely relevant to the model with VIP scores just above 1, but each saw a slight increase in the number of sites reporting jellyfish when the water was more oxygenated, as well as later in the year.

Table 2.1. Important (VIP >1) and relevant (VIP 1-1.5) variables contributing significantly to the variation in the number of sites reporting jellyfish in the Upper, Mid and Lower Estuary and their associated regression coefficients

Location	Variable	VIP - LV1	VIP - LV2	Regression Coefficient
Upper	Salinity	1.84	1.73	+0.24
Estuary	Specific Conductance at 25 °C	1.84	1.73	+0.25
	Secchi Disk Depth	1.30	1.22	+0.17
	O_2	1.03	1.04	+0.14
	Month	1.07	0.99	+0.14
Mid	Salinity	1.80		+0.08
Estuary	Turbidity	1.42		-0.07
	Month	1.27		+0.06
	pH	1.17		+0.06
Lower	Year	1.45		+0.03
Estuary	Turbidity	1.39		-0.03
	Total Phosphorous	1.20		-0.03
	Total Nitrogen	1.12		-0.03
	Salinity	1.08		+0.02

In the Mid and Lower Estuary, single latent variables explained 30 and 35% of the variation in the predictors respectively. However, the models only accounted for 12 and 5% of the variation in the outcome variable respectively. This small amount of variation explained by the two models, combined with the small regression coefficients (<0.1), indicates that despite their significance, the actual effect of these variables on the presence or absence of *C. mosaicus* in the mid estuary was limited. Within the Mid Estuary, medusae were only observed at salinities >10 except for one occasion, where they were observed at two sites. They were also more frequently observed when 'Turbidity' was low and from October to December. Within the Lower Estuary, medusae were more frequently observed in the latter months of the year when the waters were less turbid and more saline. The full description of all collected and analysed environmental variables, PLSR analysis and results table is located in Appendix 1.

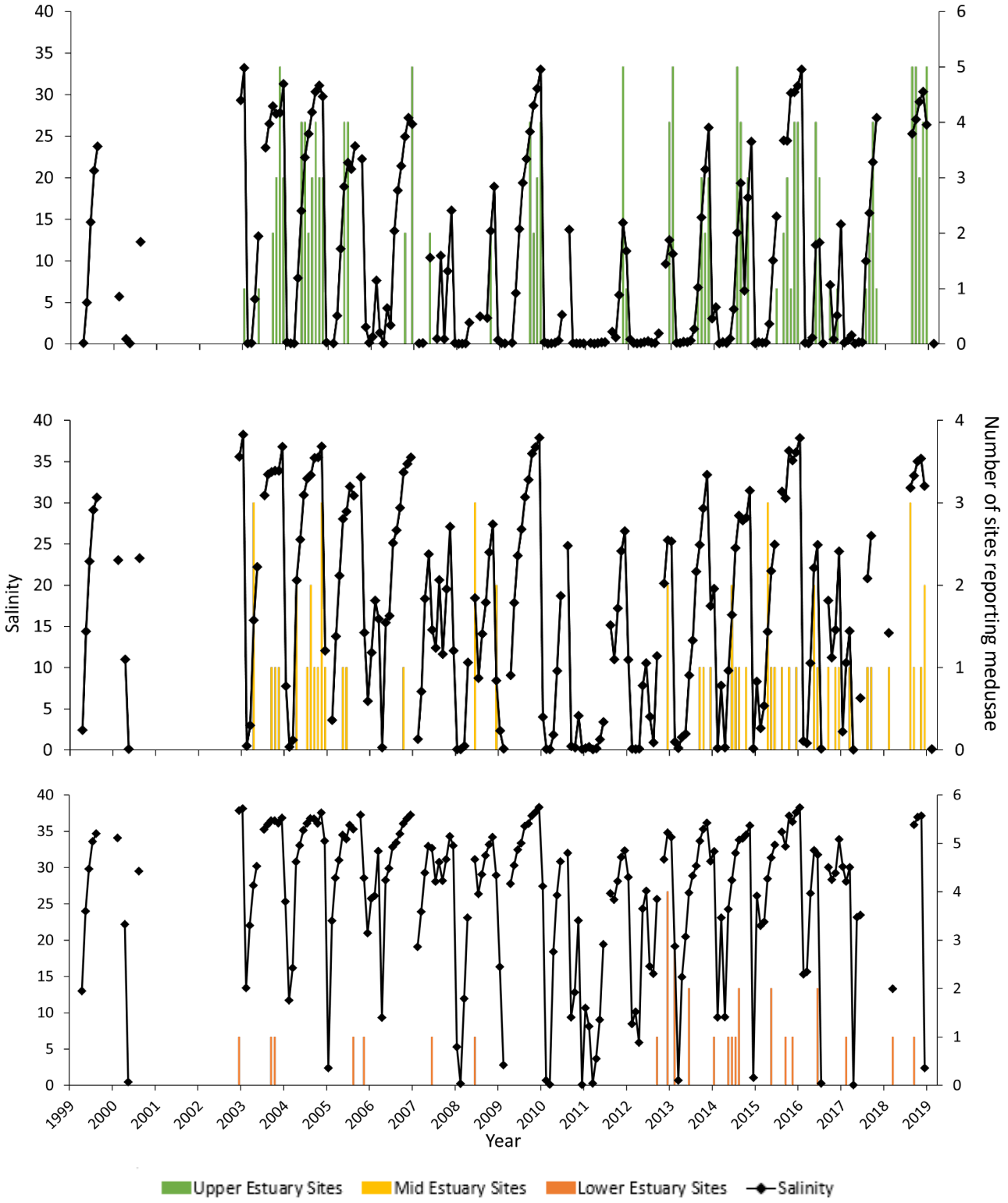

Figure 2.2. Salinity and number of Upper- (5 sites max), Mid- (4 sites max) and Lower- (6 sites max). Coloured bars represent the number of sites reporting medusae within each area of the estuary across the 20-year dataset.

2.4.2 **Prolonged Freshwater Event**

All medusae in the 34.5 (control) and 30 treatments survived and swam actively throughout the experiment. By Day 5, all the medusae exposed to a salinity of 10 ceased to pulse, and at 20 only one medusa was alive from Day 7 until the end of the experiment.

On Day 0 pulsation rates were similar across all treatments ($p>0.05$, Figure 2.3). By Day 2, medusae at 10 pulsed significantly slower than the other treatments ($F_{(3,12)}=32.241$, $p<0.001$) and on Day 3, medusae at both 10 and 20 salinity pulsed more slowly than the controls ($F_{(3,12)}=22.49$, $p<0.001$). Pulsation rates did not differ between the control and 20 treatment at any other time during the experiment ($p>0.05$).

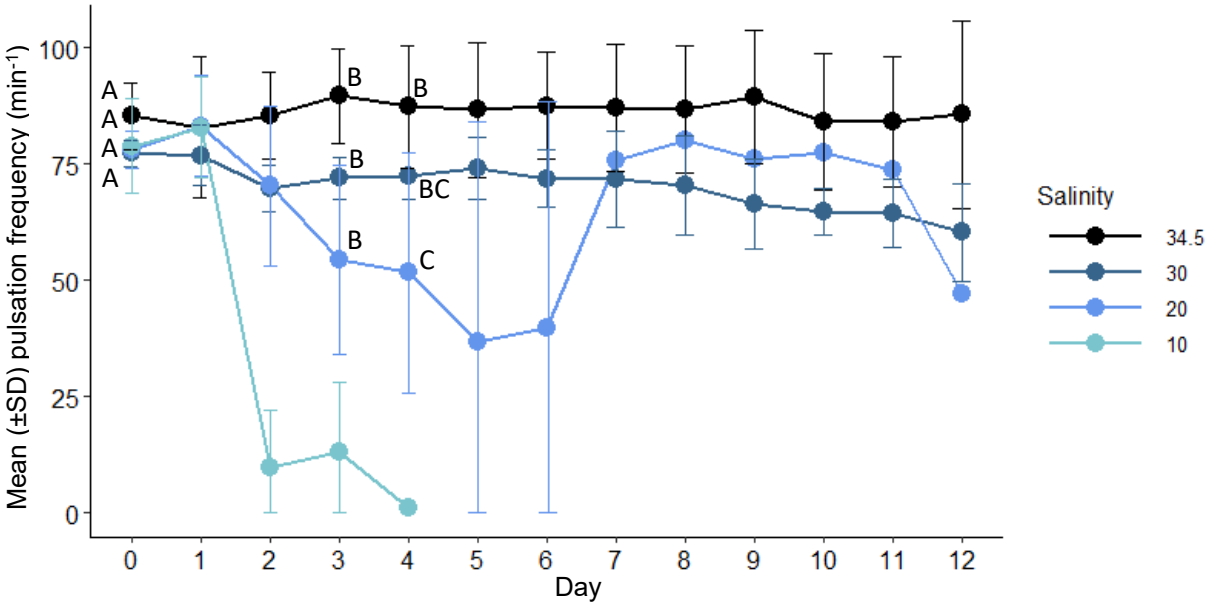

Figure 2.3. Mean pulsation frequency across the experiment (n= 4). Salinity was reduced on Day 1 after the observation. From Day 7, only 1 medusa was alive at 20. Letters above data points indicate similarities (e.g. A, A), and differences (e.g. A, B) between treatments at single time points, as determined by post hoc tests

On Day 0 respiration rates were similar across all treatments ($p>0.05$, Figure 2.4). By Day 2, medusae consumed significantly less oxygen at 10 than at control or 20 salinities ($F_{(3,12)}=3.63$, $p<0.05$). Respiration rates were similar for medusae at 34.5 and 30 on Day 7, but by Day 12 medusae at 30 had lower respiration rates than the controls ($F_{(4.39)}=-3.6241$, $p<0.05$).

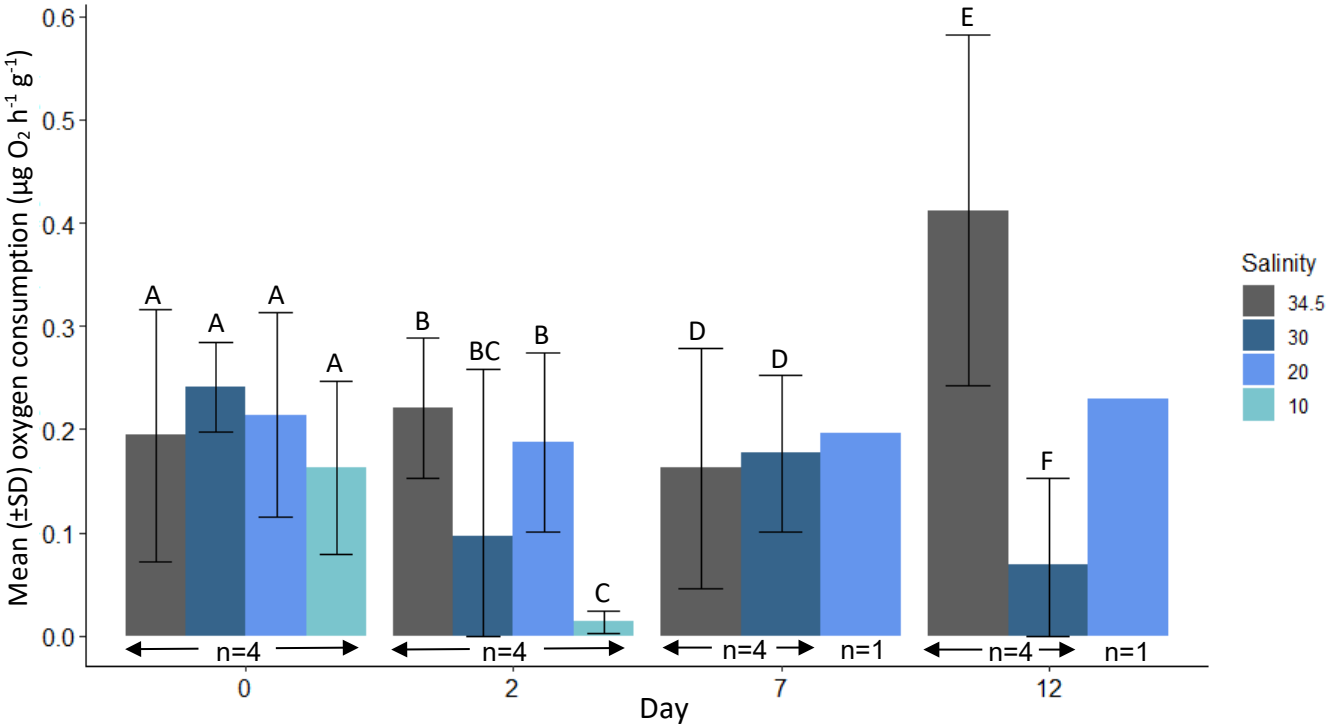

Figure 2.4. Mean (± SD) oxygen consumption across the experiment. Letters above data points indicate similarities (e.g. A, A) and differences (e.g. A, B) between treatments at single time points, as determined by post hoc tests

2.4.3 **Transient Freshwater Event**

All control medusae maintained at 33 and those temporarily exposed to 20 survived to the end of the experiment. Only 50% of medusae that experienced a temporary reduction in salinity to 10 and 15 were still alive by Day 4 and Day 17 respectively.

Prior to the decrease in salinity that occurred after the observation on Day 1, pulsation rates were similar across all treatments ($p>0.05$, Figure 2.5). On Day 2, all medusae in treatments that experienced a salinity decrease pulsed significantly slower than the controls ($F_{(3,12)}=7.965$, $p<0.01$). Following the return to ambient salinity on Day 7, medusae in treatments that decreased to 15 were still pulsing significantly slower than the controls and the treatment that decreased to 20 ($F_{(2,4.88)}=24.52$, $p<0.01$). Salinity negatively affected pulsation rates of medusae that experienced a decrease to 20, although this effect depended on the day of the experiment ($F_{(18,108)}=2.520$, $p<0.01$). From Day 2 to Day 5 pulsation rates were significantly slower in the 20 salinity treatment compared to the controls.

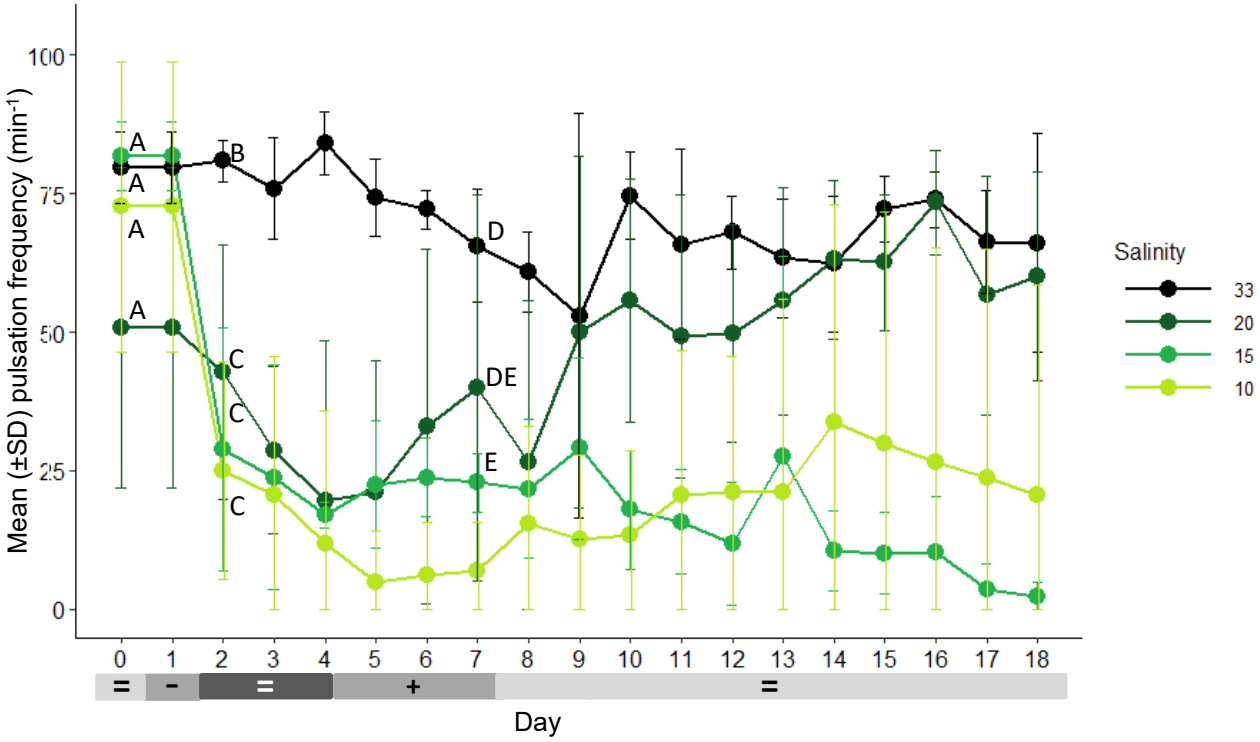

Figure 2.5. Mean pulsation frequency across the experiment (n= 4). Letters above data points indicate similarities (e.g. A, A) and differences (e.g. A, B) between treatments, as determined by post hoc tests. The grey bar indicates the direction of salinity changes across the experiment

Oxygen consumption was similar between all treatments on Day 0, 3 and 8 (excluding 10 treatment on day 8, $p>0.05$, Figure 2.6). On days 13 and 18 the controls and medusae that experienced a decrease to 20 had similar respiration rates ($p>0.05$).

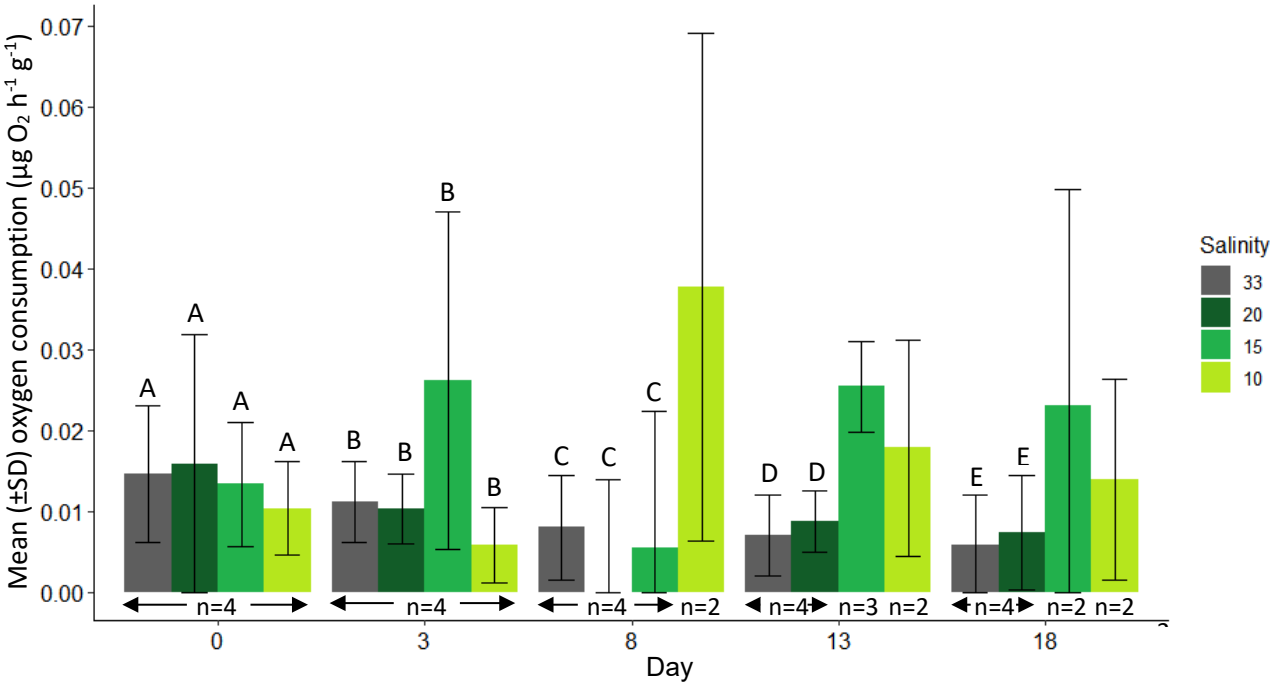

Figure 2.6. Mean oxygen consumption across the experiment (n=4). Letters above data points indicate similarities (e.g. A, A) and differences (e.g. A, B) between treatments, as determined by post hoc tests

2.5 Discussion

Barrages are commonly constructed to regulate freshwater flows in estuaries (Lehner et al., 2011; Mulligan et al., 2020). How barrages modify both the physical characters of estuaries (Ma et al., 2019; Kang and Lee, 2020), and population dynamics of estuarine animals (Amorim et al., 2018; Kim and Kim, 2020; Gillanders and Kingsford, 2002), is a key research focus with the aim of balancing societal needs with ecological impacts. Despite general investigations into how modified freshwater inputs into estuaries affect gelatinous zooplankton (Wang et al., 2016; Baumsteiger et al., 2018), we demonstrated for the first time that modification of freshwater flow by a barrage influences the population dynamics of the estuarine jellyfish *Catostylus mosaicus*. Two other studies have examined the effects of major dams and regulated flow on estuarine scyphozoan medusae (Xian et al., 2005; Amorim et al., 2018), but dams differ from barrages because their larger freshwater reservoirs have greater storage capacity and can better buffer against increased freshwater flow. Medusae were unable to survive extended periods at 10 salinity, such that they would experience when a barrage opens, but were able to survive and recover from short-term freshwater inputs such that they would experience during a moderate rainfall event that did not require substantial amounts of freshwater to be released. Consequently, artificial modifications of flow that occur following the construction of barrages are likely to result in large changes to estuarine medusa population dynamics.

Jellyfish are osmoconformers, unable to respond to rapid changes in salinity without shrinking or swelling (Albert, 2014). In an estuary characterised by sudden freshwater inputs, due to seasonal heavy rainfall and barrage openings, substantial and abrupt decreases in salinity are frequent (Margvelashvili et al., 2003). In this study, the disappearance of medusae from the upper estuary always coincided with sustained reductions in salinity. It is unclear, however, whether the freshwater discharged killed the medusae or whether they may have been flushed downstream. The two experiments we conducted provided insight into the ability of *Catostylus* medusae to withstand changes in salinity. Unlike polyps, medusae can actively move away from low salinity areas to prevent harm, with *A. labiata* observed swimming vertically downwards in response to encountering low salinity surface waters (Albert, 2012). The data presented here contained monthly observations of the occurrence of medusae at sites along the estuary, so changes in distribution along the estuary at a time scale appropriate for identifying a net downstream movement could not be identified. During periods of very high flow, salinity in the Fitzroy River can be reduced to zero 30 km below the barrage, halfway along the estuary towards the mouth. Fewer sites reported medusae when salinity was lower than 10 and, in the laboratory, severe impairment of medusae pulsation and respiration at moderate salinities was followed by death at 10 salinity, suggesting that medusae cannot survive at extreme low salinities for more than a few days to a week. Such rapid changes probably do not allow sufficient time for medusae to escape low salinity areas before they incur irreversible damage or perish. This is in line with observations that medusae are not observed in salinities <12 (Pitt and Kingsford, 2003a).

Records and anecdotal observations have reported that estuarine jellyfish are more often observed at higher salinities and disappear from estuaries following freshwater inputs (Amorim et al., 2018; Kingsford et al., 2000). Small freshwater inputs into the estuary, however, such as rainfall events, may result in less dense freshwater sitting as a lens at the surface. This may create a refuge below the halocline in which medusae can shelter during the event (Rippingale and Kelly, 1995; Albert, 2014). However, below the Fitzroy Barrage, river heights during non-flood periods are regularly <6 m, with flood events increasing the height of the river to >8-10 m (BOM, 2021a). With flood events frequently discharging >2000 m^3s^{-1} of freshwater into the estuary, it is unlikely that these deep water salinity refuges exist during flood events (Webster et al., 2003). Despite not having any refuge from osmotic changes in the rainfall simulation experiment, within a few days of recovery to marine conditions, all medusae at 20 survived and were able to recover to pre-input pulsation rates. This contrasts with the barrage opening simulation, where ¾ of medusae died within 7 days when continuously exposed to 20 salinity. In the lower part of the estuary, where salinity reductions may not be as abrupt, medusae are likely to survive rainfall events, or barrage openings if >30km downstream, by escaping to a deep water salinity refuge or outlasting

the osmotic changes. Alternatively, in response to freshwater input, medusae may have swum vertically downwards, into deeper, more turbid areas, and not been observed during sampling (Albert, 2012).

Salinity tolerances normally constrain estuarine inhabitants to specific areas of the natural salinity gradient in an estuary (Gillanders and Kingsford, 2002). Construction of a barrage greatly narrows, and in some cases, eliminates the gradient from fresh to saltwater as these constructions primarily act as a physical barrier separating fresh river water from the marine saltwater (Kim and Kim, 2020). In the Fitzroy River, the construction of a barrage greatly shortened the extent of the estuary, reducing the available habitat for medusae (Margvelashvili et al., 2003). This is not the case for all estuaries, for example in the Yangtze River, intrusion of marine water into the estuary extended further upstream after the construction of the Three Gorges Dam facilitating the introduction of marine invasive species (Xian et al., 2005). In both cases, the artificial construction determined the timing and scale of large salinity changes in their respective estuaries by releasing freshwater. In some regions, freshwater releases from dams, termed “freshets” have been posited as a way to control blooms of *Aurelia aurita* in estuaries (Amorim et al., 2018) and our data confirms that medusae are vulnerable to sudden, extreme salinity changes.

Managing freshwater flows into estuaries modified by barrages or dams should be done in a way that minimises impacts to native estuarine species, although environmental needs must be balanced with socio-economic requirements, particularly when barrages are used to mitigate flooding and protect lives and infrastructure. In an estuary dominated by freshwater inputs such as the Fitzroy, water releases could be scheduled to reduce the impact on the natural ecosystem, for example by releasing smaller volumes of water on a more regular basis to dampen salinity fluctuations in the upper estuary. As demonstrated by our “transient freshwater event” experiment, smaller releases increase the likelihood of *C. mosaicus* surviving and recovering from freshwater inputs, and would likely also benefit other native species such as the mat-forming mussel *Amygdalum* cf. *glaberrima* that occur exclusively in the upper estuary (Currie, 2005). This approach could mitigate against small to moderate rainfall events, however, this may not be possible during periods of heavy rainfall when large quantities of freshwater enter the river quickly (Webster et al., 2003). Indeed, during severe rainfall, salinity in the upper estuary would probably be reduced to 0, regardless of whether a barrage was present. The use of a barrage, however, probably increases the frequency and alters the timing of natural freshwater flushing, thereby altering the natural population dynamics of species.

Within shallow semi-enclosed systems, areas of higher salinity in deep water may provide sedentary polyps with a refuge from large seasonal freshwater inputs, enabling them to

repopulate the estuary with medusae in the spring (Rippingale and Kelly, 1995). However, this is unlikely to be the case when large volumes of freshwater are released into estuaries. For example, most barrage openings resulted in the Upper Fitzroy estuary salinity dropping to below 5 and to 0 in many cases. Whilst polyps from coastal and estuarine species appear to be more tolerant of low salinity than medusae, with some able to survive in salinities of 10 (Dong et al., 2015a; Holst and Jarms, 2010), reduced rates of asexual budding have been reported at 5 (Purcell et al., 1999), and polyps have died when salinity was reduced to 10 and <7.5, or when salinity was reduced rapidly to extreme low salinities (Holst and Jarms, 2010; Dong et al., 2015a). Whether *C. mosaicus* polyps are able to tolerate these salinity changes is as of yet unknown. Moreover, during periods of high freshwater flows following the opening of the barrage, large quantities of sediment are flushed out of the estuary (Margvelashvili et al., 2003) which is probably inhospitable to polyp colonies. The reappearance of medusae in the estuary coincides with the return of marine conditions, consistent with the exchange time for the estuary water of 100 days (Dobbie et al., 2003). This observation indicates that medusae in the estuary may be restocked from coastal populations rather than from local populations of polyps. Data on sizes of medusae, however, would be needed to help interpret recruitment patterns.

The most robust test of how modification of environmental flows affects medusae populations would require comparing the population dynamics of medusae in a modified estuary with one or more control estuaries where flows were unmodified. Comparing population dynamics of medusae in the estuary before and after the construction of the barrage would also be very informative. However, no data on the population dynamics of medusae are available from before the barrage was constructed and estuaries to the north and south of the Fitzroy River lack populations of *C. mosaicus*. Hence robust controls were not available and our conclusions from the data set are essentially based on correlations. Relying solely on correlational and circumstantial evidence to support inferences risks drawing the wrong conclusions from data (Pitt et al., 2018). Nevertheless, there are two reasons we are confident in our conclusions. First is the consistency of the pattern observed over the duration of the 20-year data set. Major flow events occurred approximately 16 times over the 20-year period and medusae disappeared from the Upper estuary following every major flow event. Secondly, our observations were supported by robust laboratory experiments, replicating changes that would occur naturally (i.e. rainfall) and as a result of estuarine modifications (i.e. a barrage opening).

2.6 Conclusions

Our study provides evidence that the population dynamics of the estuarine medusa *Catostylus mosaicus* are largely driven by freshwater inputs into an artificially modified and managed

estuary. Medusae are unable to survive extreme low salinity such as when a barrage releases large volumes of freshwater, but have significantly higher survival and recovery rates following smaller, transient changes to salinity that might occur following a rainfall event. Modifications of freshwater flows in estuaries are, therefore, likely to lead to significant changes in the population dynamics of estuarine medusae.

Chapter 3 Influence of *in situ* temperature and maternal provisioning on the medusa-to-polyp transition in a year-round population of the scyphozoan *Aurelia aurita*

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Key Words: Jellyfish; Scyphozoa; Planula Larva; Medusa; Polyp; Maternal Provisioning; Temperature; Marine Heatwave; Seasonality

3.1 Abstract

We investigated how environmental conditions translate into reproductive success or failure in *Aurelia aurita* from the medusa to the polyp life stage. This study examined how: (i), settlement success and growth of planula larvae and polyps vary across the year, (ii) the role of temperature in determining the successful settlement of larvae and growth of polyps, and iii) the role of maternal condition, provisioning and group settlement in determining the successful settlement of larvae and growth of polyps. Medusae were collected monthly from February to December 2019 from Horsea Lake, UK. Planula larvae were settled individually and in groups of 10, in conditions mimicking the *in situ* temperature and salinity of collection. For the individual treatments, planula collected in August settled most rapidly. Early growth rates were significantly higher than later growth rates and were positively correlated with temperature, unlike later growth rates. Planula length varied significantly, indicating that maternal provisioning varied across the year. July 2019 experienced a temperature anomaly, increasing the time spent by planula larvae in the water column. Increasing temperatures past thermal limits through the increasing occurrence of temperature anomalies is likely to be detrimental to larval settlement and indirectly to the replenishment of temperate polyp populations.

3.2 Introduction

Identifying how biological and environmental drivers influence the life cycles of scyphozoan jellyfish is key to understanding their responses to environmental variability (Brodeur et al., 2008), as well as predicting responses to large-scale climactic changes in the future, such as global warming (Belkin, 2009; Holst, 2012). Scyphozoan jellyfish are endemic in almost every temperate marine ecosystem (Dawson and Martin, 2001; Purcell et al., 2007), often displaying large inter-annual variation in population size and timing of appearance (Brodeur et al., 2008). This is in part due to their complex life cycles, typically featuring a pelagic medusa that produces planula larvae, which settle to the substrate and metamorphose into sedentary asexually-reproducing polyps (Lucas, 2001). Following environmental triggers, polyps can produce one or multiple ephyrae depending on the species and environmental conditions, which grow into adult medusae to complete the metagenic life cycle (Fuchs et al., 2014).

A recent focus on the benthic polyp has demonstrated that this life stage plays a critical role in maintaining jellyfish populations and is key to the formation of true blooms (Lucas et al., 2012; Sukhoputova and Kraus, 2017). The majority of experimental work has focused either on medusae (Albert, 2014; Minamoto et al., 2017), or polyps cultured in aquariums (Widmer et al., 2016; Webster and Lucas, 2012). In contrast to these two 'adult' life stages, the planula larva is relatively understudied; yet this short-lived juvenile life stage potentially represents a key moment in the life cycle. Successful settlement and metamorphosis of planula larvae is essential to the replenishment of benthic polyp populations, contributing directly to the success or failure of the benthic population, and indirectly to the potential development of future jellyfish blooms (Duarte et al., 2013).

Scyphozoan planulae are non-feeding, simple larvae that undergo significant metamorphosis following settlement (Pechenik, 1999). They respond to light (Svane and Dolmer, 1995), temperature (Gambill et al., 2018) and gravity, as well as to chemical and tactile stimuli (Tomaru et al., 2014; Yoon et al., 2014) such as salinity (Conley and Uye, 2015; Dong et al., 2018; Takao and Uye, 2018), pH (Dong and Sun, 2018) and other factors (Young and Chia, 1981; Ishii et al., 2008). Settlement and growth has been examined in several species, such as *Cyanea capillata* (Brewer, 1976b; Holst and Jarms, 2010), *Cyanea lamarckii*, *Chrysaora hysoscella* and *Aurelia aurita* (Holst and Jarms, 2007; Purcell et al., 2009). *Aurelia aurita* planula larvae demonstrate an increased rate of metamorphosis in the presence of established conspecifics (Gröndahl, 1989); although others attribute aggregated settlement to confounding factors such as hydrodynamics (Keen, 1987). Due to their small size and short lifespan, research on planula larvae settlement rates, preferences and

behaviour has largely been confined to laboratory studies examining this life stage independently from the others.

As the planula larva is a non-feeding stage, adequate maternal provision is key to the success of the larva (Lucas and Lawes, 1998; Wendt, 2000). Without sufficient provisioning, the larvae are likely to die before settlement, or be forced to choose a suboptimal settlement location (Marshall and Keogh, 2003). Additionally, release in sub-optimal conditions may also result in larval death and endanger the future of the benthic population. Once released from the medusa, planulae are estimated to have enough energy to survive in the water column for a few days to a week at 20°C (Schneider and Weisse, 1985), however laboratory experiments have demonstrated that planulae can survive for up to three weeks (Conley and Uye, 2015). In *C. lamarckii*, increased settlement temperatures between 9.4 and 26.8 °C were been linked to decreased settlement time (Gambill et al., 2018); however, in *Aurelia aurita* from southern UK, settlement success was found to decline at increased temperatures of 18°C as compared to 6 °C (Webster and Lucas, 2012). This highlights the species and population-specific thermal tolerance limits influencing the larval stages (Riascos et al., 2013; Goldstein et al., 2017).

Investigating the scyphozoan life cycle as a whole illustrates how each life stage can affect the overall population dynamics (Goldstein and Steiner, 2020). However, most experiments typically study each life stage in isolation (Sukhoputova and Kraus, 2017). We therefore chose to embed our experimental procedure firmly in a realistic ecological context by sampling medusae across a year, and determining the parameters of our experiments using these data. Our unique blend of *in situ* and laboratory experiment allows us to take into account changing environmental conditions and replicate these under controlled conditions in the laboratory. This study focuses on clarifying how timing and temperature at larval release impacts on the quality of the reproductive output, including settlement rate, success, and larvae survivorship. Through this, a more complete picture of how ambient conditions translates into reproductive success or failure can be obtained. We hypothesise that: (i) settlement success and growth of planula larvae and polyps varies across the year; (ii) environmental factors such as temperature will play a direct role in determining the successful settlement of larvae and growth of polyps across the year, and iii) other factors such as maternal condition, provisioning and group settlement will play an indirect role in determining the successful settlement of larvae and growth of polyps across the year. We also examine the case of temperature anomalies, specifically abnormally high temperatures, and the role these may play in determining the success of a population.

3.3 Methods

3.3.1 Medusae Collection

Specimens of *Aurelia aurita* were collected monthly from Horsea Lake, UK (50°49'58.8; -1°05'36.9) from February-December 2019. Horsea Lake is a brackish, semi-enclosed, man-made body of water connected to Portsmouth Harbour via a controlled pipe and valve, and the bottom (6 m) water temperature typically ranges from 5.5 °C in February to 23.0 °C in July (Lucas, 1996; CEFAS, 2018).

Medusae were collected on 10 occasions throughout 2019 (Table 3.1). On each occasion, any specimens visible from the dockside were collected using a net and a bucket. Temperature and salinity were measured at the surface at time of collection.

Table 3.1. Sample dates and information on collected medusae

Sample Date	Temperature (°C)	Salinity	Total Medusae Collected	Mean Diameter mm (±SE)	Mean Wet Weight g (±SE)	Gravid Medusae Collected
06/02/2019	6.0	22.0	55	66.76 (±2.26)	16.04 (±1.36)	23
21/03/2019	10.6	24.1	35	62.49 (±4.32)	14.41 (±4.17)	18
26/04/2019	13.4	24.5	3	71.67 (±27.55)	27.57 (±23.00)	2
29/05/2019	17.6	25.3	2	63.50 (±12.50)	14.11 (±8.66)	1
26/06/2019	20.5	25.0	2	102.50 (±15.50)	53.83 (±26.69)	0
24/07/2019	24.1	25.0	13	72.46 (±9.54)	22.91 (±7.15)	5
03/09/2019	20.8	27.5	131	80.52 (±2.99)	35.27 (±5.36)	8
25/09/2019	18.1	24.0	9	110.11 (±20.40)	100.22 (±53.03)	3
22/10/2019	13.7	23.7	37	99.41 (±7.19)	62.95 (±14.27)	12
12/12/2019	9.3	23.1	12	174.25 (±13.51)	273.30 (±43.87)	4

On each occasion, medusae were immediately brought back to the National Oceanography Centre Southampton, where sex, diameter (mm), wet weight (g), and condition (inverted bell, degraded bell edge, missing or additional gonads/oral arms) were recorded. Planula larvae from sexually mature female medusae were collected into a single beaker by rubbing the oral arms, and set aside for the settlement experiments. Two gonads and two oral arms were dissected whole out of the female specimens and stored in 4% formalin for histological analysis.

3.3.2 **Experimental setup**

Prior to the start of each settlement experiment, eight six-well plates and ten 10 mL Pyrex beakers were preconditioned for at least 24 hrs in seawater to allow the development of a bacterial biofilm. Both individual wells and group beakers were filled with 10 mL seawater from Horsea Lake, collected at the same time as the medusae and filtered through a 1µm mesh. Individual larvae were placed in each well of the six-well plates (n=48) and groups of 10 larvae were introduced into each 10 ml beaker (n=10, total=100).

Developmental stage was recorded for both individual and group experiments every 1-3 days (dead, swimming larvae or settled polyp with the number of developed tentacles) using an optic microscope. The total number of planulae/polyps at each developmental stage was recorded for the group beakers as it was not possible to track individuals. Seawater was refreshed every 5-6 days with filtered seawater of the same salinity and temperature across the experimental period and polyps were fed one 1-day old *Artemia* nauplius roughly every 1-3 days once the first tentacles had grown. The monthly experiments were stopped once all polyps had reached 16 tentacles or had died (31-104 days). The October experiment was stopped early on day 52 when polyps were still developing for logistical reasons.

3.3.3 **Histology**

Dissected gonads and oral arms were dehydrated in graduated isopropanol baths and embedded in paraffin wax, before being sliced into 7µm sections using a microtome. Sections were stained using Haematoxylin and Eosin (Avwioro, 2011; Alturkistani et al., 2016) and photographed using a stereomicroscope before being analysed in ImageJ. Where possible, 50 random oocytes were measured for each female medusa, with each individual oocyte measured four times to determine average ferret diameter.

3.3.4 **Planula larvae**

The oral arms stored in 4% formalin were removed and gently rubbed to release any larvae. Fifty random planula larvae were measured for each female medusa, along the longest axis of the larva.

3.3.5 **Statistical analysis**

All data analysis was carried out in R V4.0.3. Only individually settled replicates were analysed unless otherwise stated. All data were analysed for normality using Shapiro-Wilk tests and QQ plots, and non-parametric tests were carried out if transformation was not sufficient.

Table 3.2. Hypotheses and associated statistical tests

Hypothesis	**Statistical Test**
Planula larva mortality ~ Month	Logistic regression GLM (binary data: alive/dead)
Nbr. days spent in water column ~ Condition (alive/dead)	One-way ANOVA using cube-transformed data
Nbr. days spent in water column ~ Month	One-way ANOVA using cube-transformed data, post hoc Tukey HSD
Nbr. tentacles produced per day (up to 8 tentacles) ~ Month	Kruskal Wallis rank sum test, post hoc pairwise comparisons
Nbr. tentacles produced per day (8-16 tentacles) ~ Month	Kruskal Wallis rank sum test, post hoc pairwise comparisons
Nbr tentacles produced per day ~ development stage (0-8 vs >8-16 tentacles)	Wilcoxon rank test
Mean maximum tentacle nbr. ~ Month	Kruskal Wallis rank sum test, post hoc pairwise comparisons
Nbr. tentacles produced per day (up to 8 tentacles) ~ Temperature	Kruskal Wallis rank sum test, post hoc pairwise comparisons
Nbr. tentacles produced per day (8-16 tentacles) ~ Temperature	Kruskal Wallis rank sum test, post hoc pairwise comparisons
Egg ferret diameter ~ Month	One-way ANOVA
Planula larva length ~ Month	Welch's ANOVA using log transformed data, post hoc Games Howell test
Mean nbr. of days to settlement ~ mean planula larvae length	Pearson Correlation
Mean tentacle number on day 6; 13; 20 ~ treatment (individual vs conspecific settlement)	Kruskal Wallis rank sum test

3.4 Results

3.4.1 Mortality

On average, 8% of individual planula larvae settled and survived to full polyp maturity (i.e., 16 tentacles) in any of the individual settlement experiments (Figure 3.1). Mortality in October cannot be fully determined as the experiment was not completed.

On average, 50% of planula larvae died before they settled, the fewest dying in May and July (18 larvae, 38% of total), and most in August (30 larvae, 63% of total). There was no significant difference in planula mortality across the year, nor in relation to temperature ($p>0.05$).

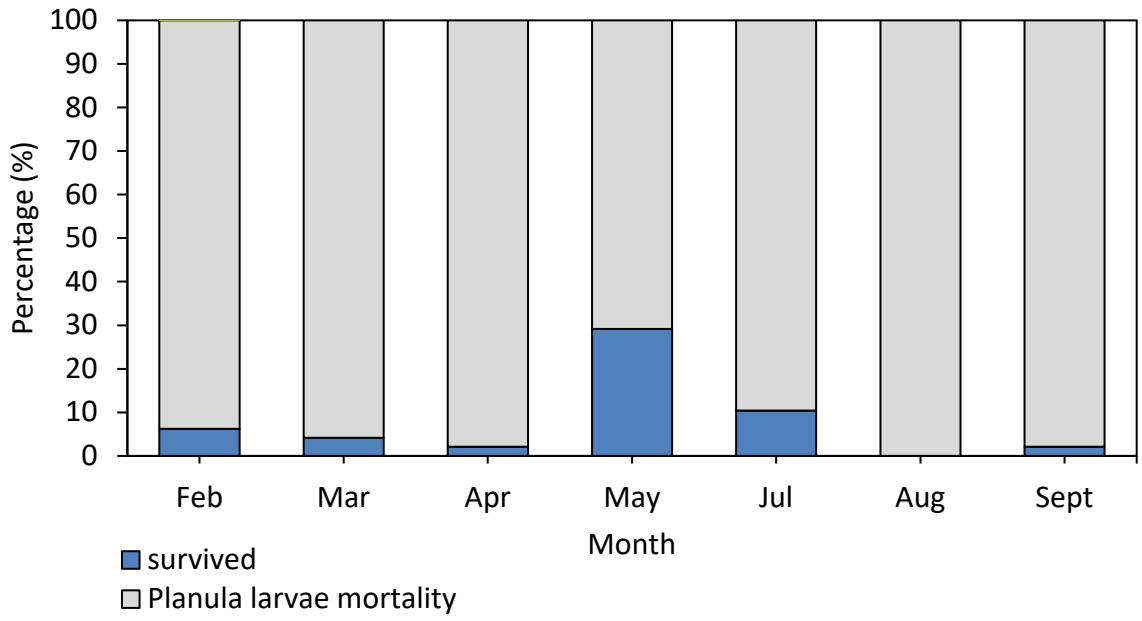

Figure 3.1. Percentage of individuals that survived to 16 tentacles, or died each month (excluding October). n=48 planula per month

3.4.2 Settlement Rate

The settlement rate differed significantly between months (Figure 3.2, one-way ANOVA, $F_{(7,376)}=13$, $p<0.001$). Planulae collected in August settled fastest, on average < 5 days ($p<0.05$). In contrast, planula larvae in July remained in the water column for ~12 days, significantly longer than all other months except for March.

The number of days that planulae spent in the water column did not differ between planulae that successfully settled and those who died before settlement ($p<0.05$), and there is no significant correlation between days to settlement and settlement success (Pearson correlation, $r=-0.02$, $p<0.05$).

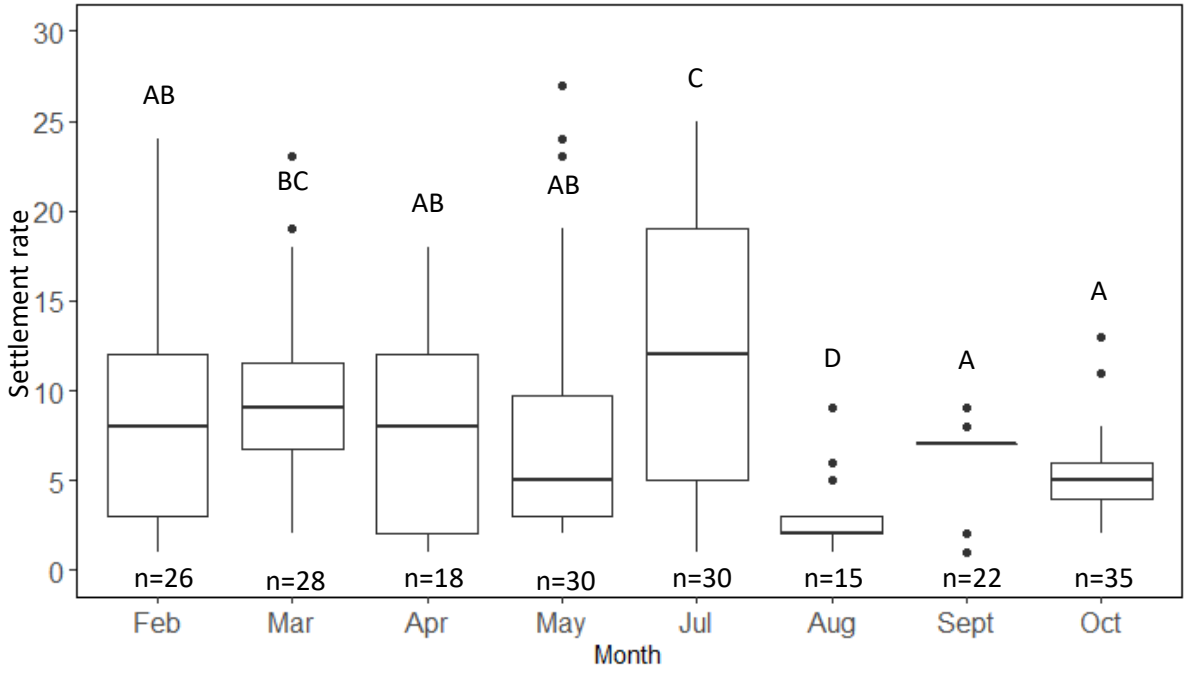

Figure 3.2. Settlement rate of individually settled larvae across each monthly settlement experiment. Letters above data points indicate similarities (e.g. A, A), and differences (e.g. A, B) between treatments, as determined by post hoc tests. For example, August is significantly different to all other months, and February is similar to all months with an A and/or a B letter, i.e. all months except for July and August. Statistical table can be found in Appendix B detailing the results further

3.4.3 **Development Rate**

Early tentacle development rate was significantly faster than the late growth rate (2.4 and 0.5 tentacles day^{-1}, respectively; Wilcoxon rank sum test with continuity correction, $W=7265.5$, $p<0.001$). There was a significant difference in early tentacle development rates between different months (Kruskal Wallis rank sum test, $chi=33.332$, $df=7$, $p<0.001$, Figure 3.3). Pairwise comparisons revealed that August had a significantly faster tentacle development rate (>4 tentacles day^{-1}) than all months except for May and September ($p>0.05$). Early tentacle development rates in May were significantly faster (~3 tentacles day^{-1}) than in all months except

for March and August. Late tentacle development rates did not vary significantly between different months (<1 tentacle day^{-1}; p>0.05).

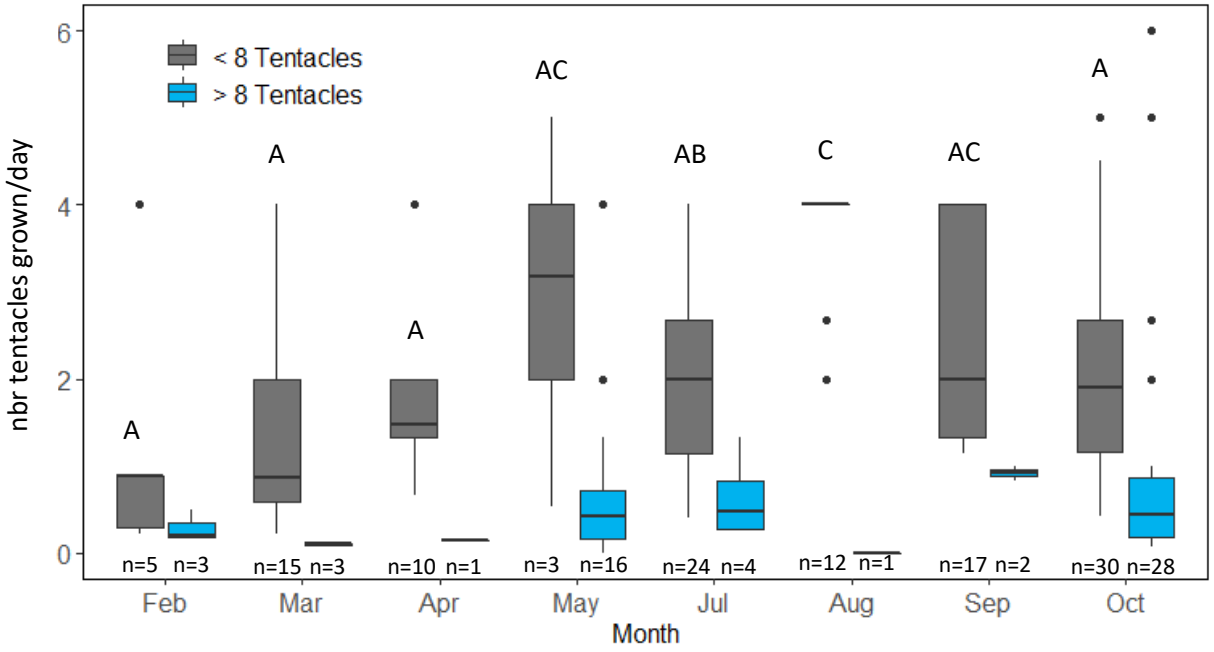

Figure 3.3. Mean daily tentacle development rate for early (<8 tentacles), and late (8 to 16 tentacles) across each monthly settlement experiment. No polyps developed 16 tentacles in August, so only the early tentacle development rate is displayed for this month. Letters above data points indicate similarities (e.g. A, A), and differences (e.g. A, B) between early tentacle development rates, as determined by post hoc tests

The average total number of tentacles grown by each polyp varied significantly between months (Kruskal Wallis rank sum test, chi=64.289, df=7, p<0.001, Figure 3.4). Polyps settled in May and October grew significantly more tentacles than any other month except for February, despite the October treatment remaining incomplete (i.e. there were still polyps that had <16 tentacles alive).

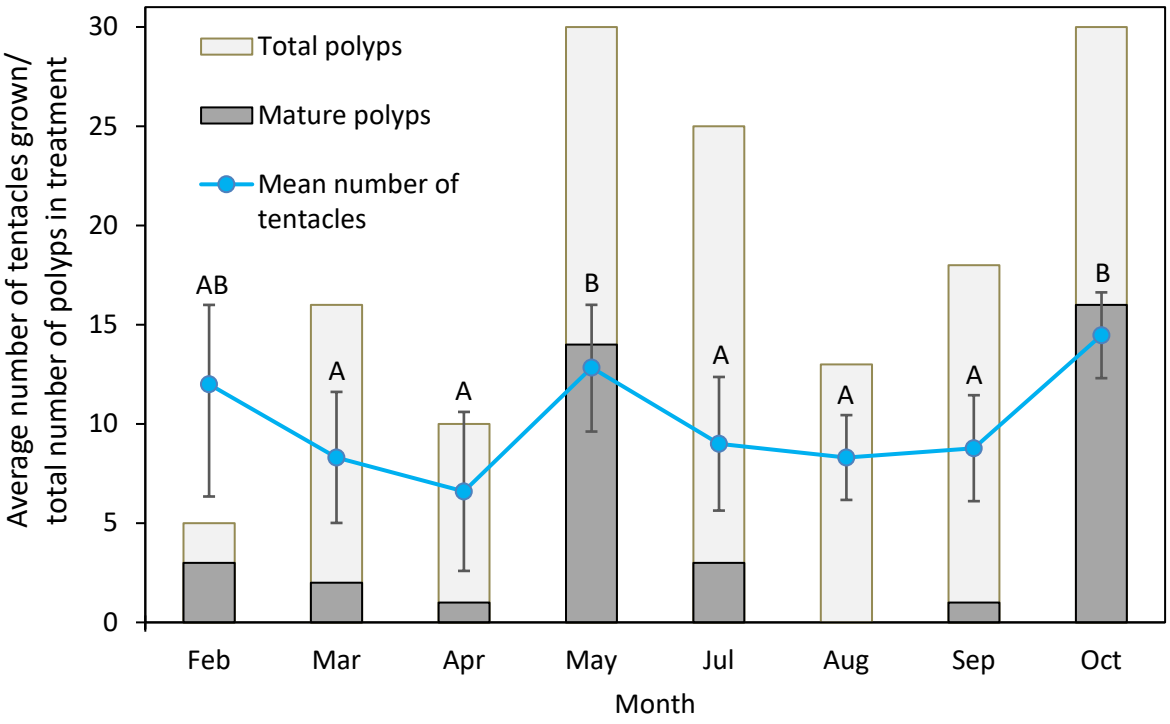

Figure 3.4. Mean (± SE) number of tentacles grown by each polyp over the course of the experiment (blue line), the total number of planulae that settled and metamorphosed into a polyp (pale grey bar), and the total number of polyps that survived to maturity at the end of each experiment (dark grey bar). Initial number of polyps = 48 total/month. Letters above data points indicate similarities (e.g. A, A), and differences (e.g. A, B) between the mean number of tentacles, as determined by post hoc tests

3.4.4 **Temperature**

Polyps incubated at higher temperatures produced tentacles at a faster rate than those maintained at low temperatures (Kruskal Wallis rank sum test, chi=33.332, df=7, $p<0.001$, Figure 3.5). Later growth rates over 8 tentacles did not increase with rising temperatures ($p>0.05$).

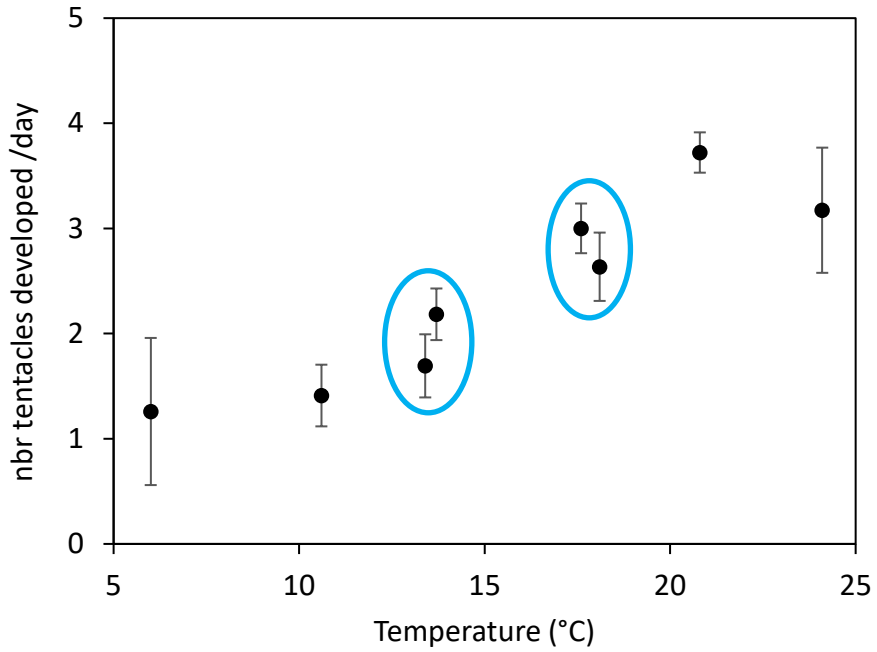

Figure 3.5. Mean daily tentacle development rates up to 8 tentacles of polyps incubated at different temperatures (±SE). Different months with similar temperatures are circled in blue.

Temperatures were broadly comparable (±1°C) at four points across the experimental period: March (10.6°C) and December (9.3°C); April (13.4°C) and October (13.7°C); May (17.6°C) and September (18.1°C); and finally June (20.5°C) and August (20.8°C). Only April and October, and May and September can be compared for logistical reasons. We note that the October treatment is incomplete so conclusions from this comparison must be considered carefully.

Early polyp growth rates (Figure 3.3) varied significantly across the sample months, indicating that temperature is likely to have a strong effect on polyp growth. However, a greater proportion of polyps grew to maturity in May (~30%), as compared to September (<5%, Figure 3.1). As well as this, in October each polyp grew on average over twice as many tentacles than in April, and in May each polyp grew on average 1.5 times as many tentacles than in September (Kruskal Wallis rank sum test, chi=64.289, df=7, p<0.001, Figure 3.4). This suggests that temperature is not the only factor influencing the successful settlement of planula larvae and polyp growth to maturity.

3.4.5 **Maternal input**

Minimum size at maturity of female medusae varied across the year, with the smallest mature medusae in April measuring 33 mm bell diameter compared with 190mm in December (Figure 3.6). Despite these differences, across all months the minimum size at maturity corresponded to the smallest female medusae collected for that month, and all but three females had either eggs

present in the gonads or planula larvae in the oral arms. Only three female medusae were collected in September, two in April and a single female was collected in May.

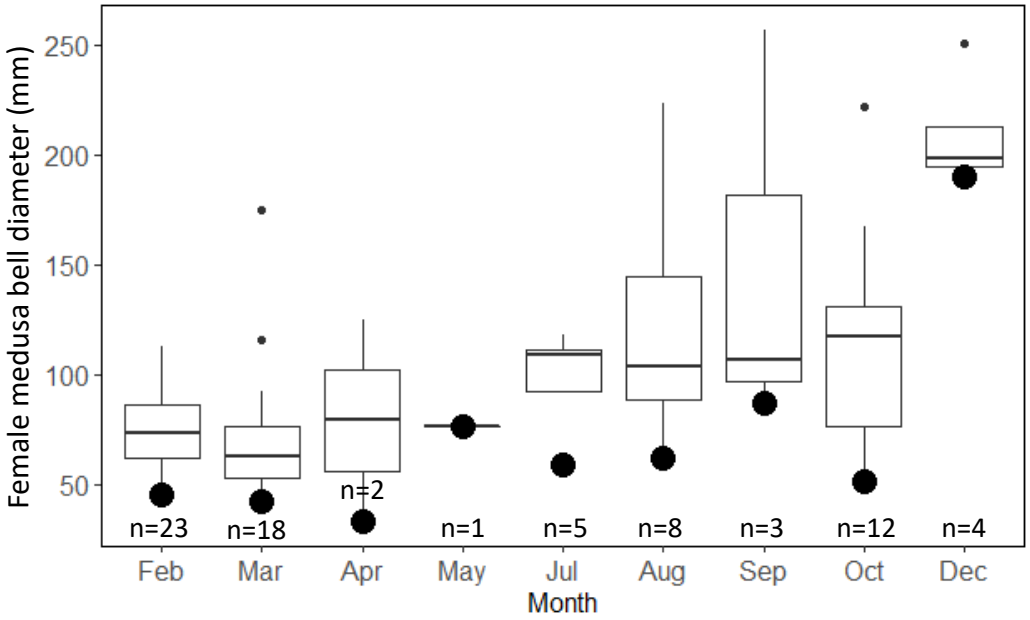

Figure 3.6. Female medusa bell diameter across 2019, with minimum size at maturity for each month represented by a black circle

Egg size varied on average between 50–60 µm, but did not vary significantly across the sample months, nor did it vary with temperature or salinity ($p<0.05$). Neither maximum nor minimum oocyte size correlates significantly with any environmental variable.

Planula larva length was significantly different across the year (Welch's ANOVA, $F_{(7,262)}=26.3$, $p<0.001$, Figure 3.7). Planula were significantly longer in May and July as compared to the rest of the year (Games Howell post hoc test, $p<0.05$), and were significantly shorter in April and September as compared to the rest of the year (Games Howell post hoc test, $p<0.05$).

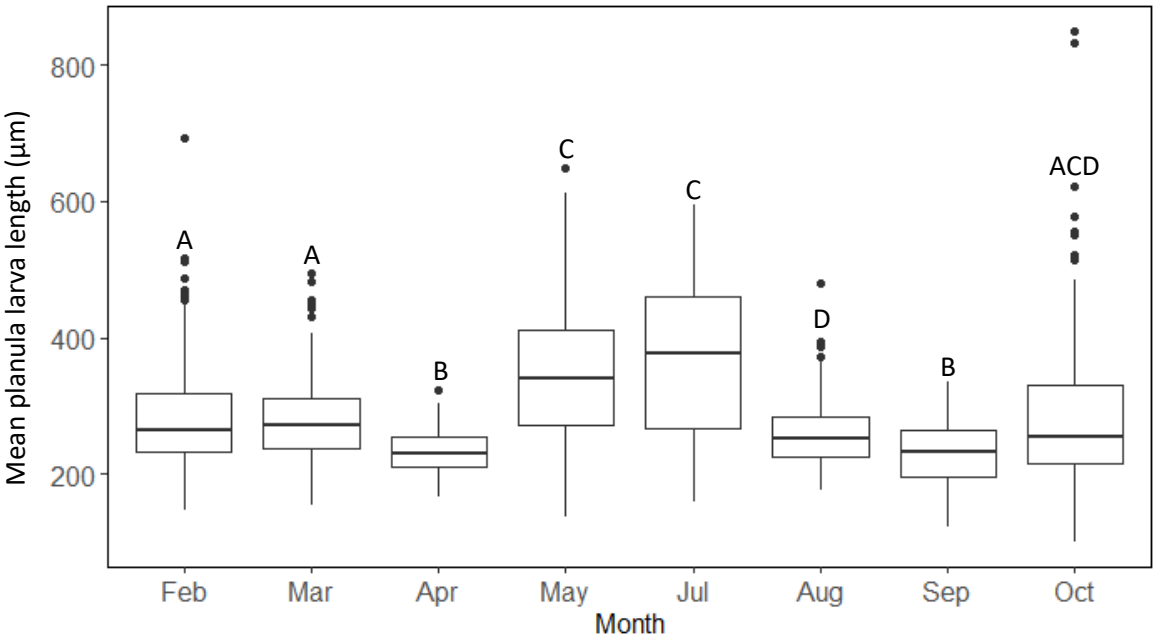

Figure 3.7. Mean planula larvae length (µm) across 2019. Box and whisker plot. Letters above data points indicate similarities (e.g. A, A), and differences (e.g. A, B) between treatments, as determined by post hoc tests. n=50

There was a moderate positive correlation between the average number of days to settlement and the mean planula larva length (Pearson correlation, r=0.56, p<0.05, Figure 3.8)

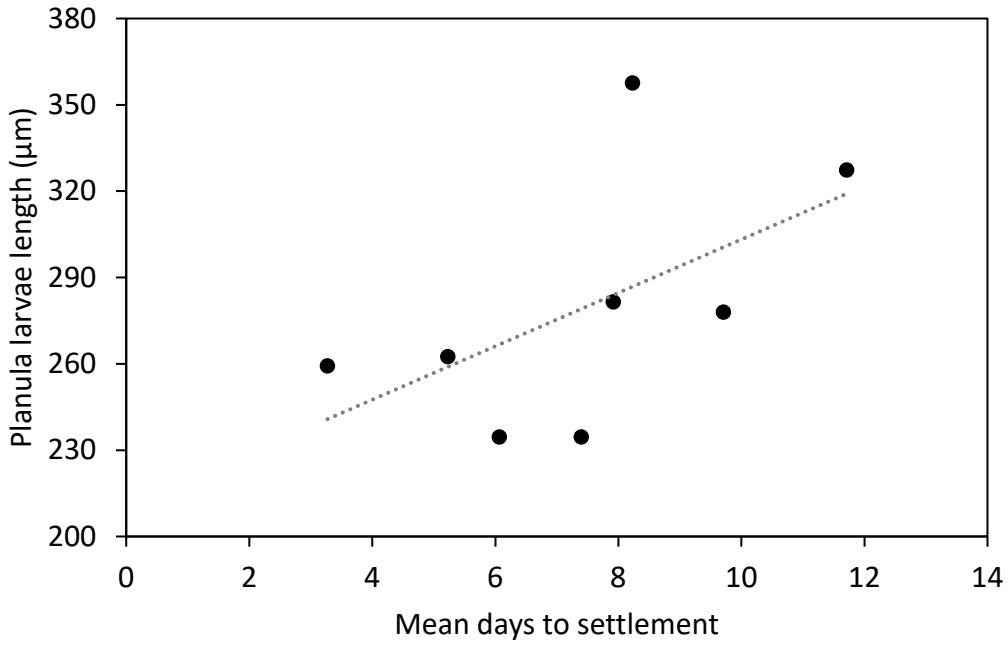

Figure 3.8. Mean planula larvae length (µm) by mean settlement rate across sample months. The dotted line indicates the positive correlation between these two variables

3.4.6 **Conspecific settlement**

Mortality was lower when settling in a group with conspecifics, with on average 16% surviving to full maturity across 2019, ranging from 28% surviving in September, to only 4% in March.

Polyps settled with conspecifics on average grew significantly more tentacles by Day 6 than polyps settled individually (Kruskal Wallis rank sum test, chi=39.153, df=1, p<0.001, Figure 3.9). However, there were no significant differences in the mean numbers of tentacles grown by Day 13 or 20 in individual and group experiments.

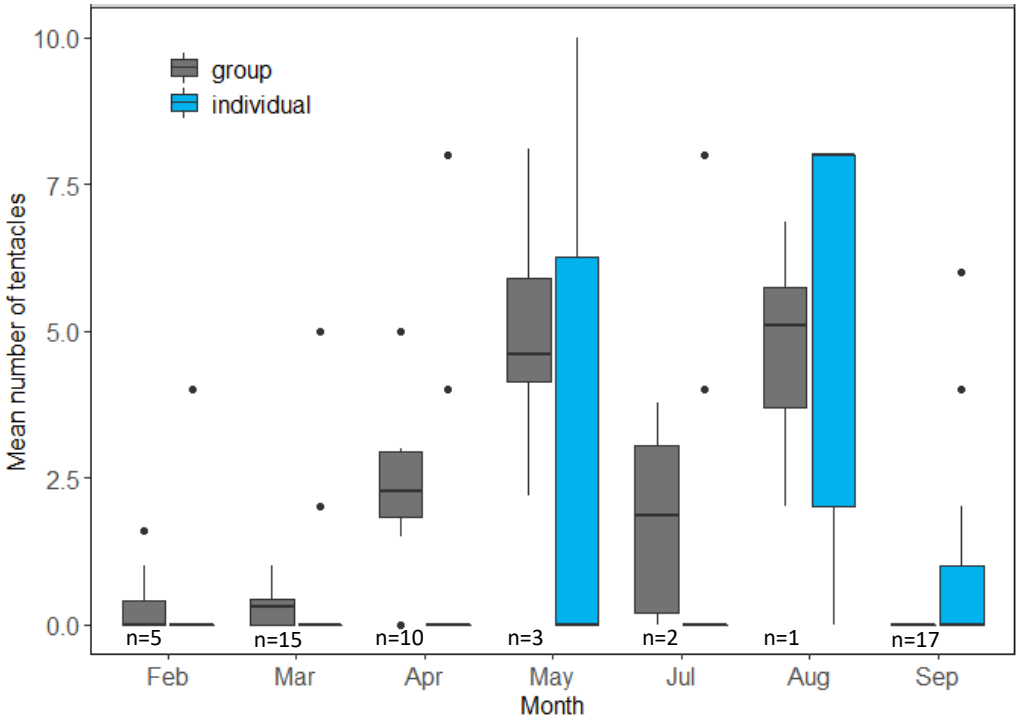

Figure 3.9. Mean number of tentacles grown by isolated (n= varied, displayed on graph) or grouped (n=10) polyps 6 days after settlement across sample months

3.5 **Discussion**

Our results suggest that temperature is a key variable influencing early tentacle development rates of newly settled *Aurelia aurita* polyps. Early growth rates of polyps (i.e. up to 8 tentacles) were positively correlated with temperature, indicating that once planulae settled, newly-formed polyps grow their first 8 tentacles more rapidly at warmer temperatures. This may enable these polyps to start feeding earlier, thus growing to a reproductive age/size more quickly and therefore replenish the benthic population more rapidly than larvae that settle and metamorphose in the cooler months of the year. Slower-growing polyps are likely to be vulnerable to overgrowth and predation (Watanabe and Ishii, 2001; Colin and Kremer, 2002), as well as to running out of

internal food stocks before maturing sufficiently. In contrast, polyp growth rates beyond 8 tentacles were not driven by temperature, and we hypothesise that this change may represent the transition point between using internal food stocks provided by the parent, and the polyp being able to catch enough of its own food.

Polyp tentacle development rates at the highest temperature (24.1 °C) were slower than those at ~20 °C. In our dataset, July 2019 appears to have been a thermal anomaly for this area, with temperatures much warmer than normally experienced on the south coast of the UK. Between 1984 and 2012 the mean temperature at Fawley power station (50°50'N, 1°20'W) in July was 18.8 °C (±1.38), with a maximum recorded temperature of 21.6 °C (CEFAS, 2018). Horsea Lake is shallow and likely to experience greater temperature extremes than coastal waters, and although previously published data from Horsea Lake note a high of 23 °C (Lucas, 1996), the temperature recorded at Horsea Lake in July 2019 (24.1 °C) was anomalously high, likely as a result of a short-term heatwave in the local area. Thermal windows constrain scyphozoan populations, limiting their geographical range (Höhn et al., 2017). Each population's thermal window differs according to the environmental conditions they experience, and this may contribute to population-specific phenology (Lucas, 1996; Dawson and Jacobs, 2001). Aerobic metabolism begins to decline once polyps reach the upper limits of their thermal window, usually any temperature reported for the area warmer than the monthly mean (Gambill and Peck, 2014; Höhn et al., 2017).

Our data indicate that the Horsea Lake *Aurelia* population may have reached its thermal limit in July 2019. This is particularly apparent when examining larval settlement. Settlement rates were on the longer side of past estimates (Schneider and Weisse, 1985), with most larvae remaining in the water column for on average a few days to just over a week. However, larvae released in July, coincident with the anomalously high temperature, spent more time in the water column than the other months. Larvae may have been reaching their thermal maxima with the water temperatures too hot for them to settle efficiently. The July temperature data may have been coincident with a short-term marine heatwave, although this cannot be confirmed without temperature records across a period of five consecutive days or more (Hobday et al., 2016). Nevertheless, heatwaves have adverse effects on marine invertebrates, including increasing the frequency of failed reproduction and affecting recruitment and population maintenance in numerous marine taxa (Shanks et al., 2019). Some temperate fouling species such as bryozoans and ascidians show a thermal tolerance in realistic experimental simulations (Smale et al., 2015). Related jellyfish species such as *A. coerulea* report negative effects of high temperatures on settlement, including smaller planula size and reduced survival rate (Dong et al., 2018). In our experiment, there were no significant differences in settlement success over the different months. Planula larvae in July were significantly larger than other months and this may have also

contributed to their longevity. On the one hand, if temperatures continue to rise as predicted (Belkin, 2009), benthic populations may suffer in the summer months as a result of delayed settlement which increases vulnerability to the environmental and to predators. On the other hand, if temperatures rise in the winter months then larvae may encounter more favourable conditions for settlement and growth, but may suffer from reduced strobilation in the spring (Loveridge et al., 2021).

R strategists such as scyphozoan medusae typically live in unstable, unpredictable environments, and high fecundity coupled with little investment in the larvae mean that offspring that survive long enough to settle are likely to settle in lots of different environments, with no guarantee of optimal growing conditions (Pechenik, 1999). Across any of the individual settlement experiments fewer than 10% of planula survived from release to polyp maturity. Increased time spent in the water column poses significant risks to the planula larvae, as they are vulnerable to predation, environmental conditions such as extreme temperatures (Gambill et al., 2018), reduced salinity (Dong and Sun, 2018), or being carried away from suitable settlement sites and other conspecifics which help to protect against predators and overgrowth (Marshall and Keogh, 2003). When settling amongst conspecifics, despite faster development across the first six days post-settlement by day 13 there were no significant differences in tentacle development. Experiments examining early polyp development should be cautious in the interpretation of their results if only examining individual mesocosms, however our results indicate that within two weeks there was no difference in tentacle development between individual and grouped treatments. Our results support previously-published experiments that temperature is a strong driving factor behind planula larva settlement and successful growth to maturity in polyps (Webster and Lucas, 2012). However, it does not explain all the variability we encountered in our dataset, and other factors must be taken into consideration, such as maternal provisioning.

Maternal provisioning did not vary across the year in terms of egg size, although planula larvae were significantly different lengths across the year, indicating that there was some variation in terms of maternal provisioning (Lucas and Lawes, 1998; Wendt, 2000). Planula larvae have a limited amount of time to find a suitable settlement surface (Marshall and Keogh, 2003). As larvae age, finding a place to settle becomes more urgent, and this could lead to less discrimination in choice of settlement surface (Knight-Jones, 1951, 1953, Gibson, 1995). Research suggests that the maximum time spent in the water column depends on not only the environment into which the larvae is released (Conley and Uye, 2015), but also on the energetic reserves provided by the parent (Schneider and Weisse, 1985; Wendt, 2000). In our study, planula in May and July were the largest, which may have been due to more abundant food available for the medusa in the spring, coupled with warmer conditions enabling faster larva growth and development. However, it has

previously been suggested that in Horsea Lake medusae may direct food resources into somatic growth when abundant, and reproductive effort when food is scarce (Lucas, 1996). There was no variation in substrate type, and in the absence of settlement cues, larger larvae may take longer to settle (Marshall and Keogh, 2003).

Adaptation to local thermal conditions is a possibility in Horsea Lake, as the *A. aurita* population there does display unusual characteristics not seen in many other populations such as the production of eggs and larvae across the year, as well as medusae being present year round (Lucas et al., 1997). These factors enable us to examine planula larvae settlement and growth to maturity in a range of realistic environmental conditions. Our results have provided us with informative responses to realistic temperatures; however, this approach is not without limitations. Highly variable medusa sample numbers between different months, as well as low sample number in some of the settled polyp groups means some of our conclusions could benefit from being verified by further experiments. This study has further illuminated the complexity of the scyphozoan life cycle and its many driving factors. Reproductive strategy, maternal provisioning and the environmental conditions all feed into a complex model that determine planula larvae settlement success and polyp growth to maturity.

3.6 Conclusions

This study focuses on clarifying how timing and temperature at larval release impacts on the quality of reproductive output, including settlement rate, success, and survivorship. Our results indicate that if studied in isolation from other life stages, increasing temperatures could appear beneficial to scyphozoan populations, by increasing early polyp growth rates. However, when put in context with other parts of the life cycle, increasing temperatures past thermal limits even in the short term through the increasing occurrence of temperature anomalies is likely to be detrimental to larval settlement and indirectly to the replenishment of temperate polyp populations. Whilst temperature remains a driving force for settlement and growth, it does not explain all of the variation that we observed in our dataset. Revisiting our hypotheses, settlement success and growth of planula larvae and polyps did vary across the year. Environmental factors such as temperature drove early polyp growth; however, other factors such as maternal provisioning and group settlement also determined the successful settlement of larvae and growth of polyps across the year. Finally, temperature anomalies may exceed the population's thermal limits and future increases due to climate change may lead to a decline in some temperate jellyfish populations.

Chapter 4 Transgenerational effects influence acclimation to a varying climate in *Aurelia aurita* polyps (Cnidaria: Scyphozoa)

Key Words: Jellyfish, Scyphozoa, Polyp, Transgenerational Acclimation, Phenotypic Plasticity, Temperature

4.1 Abstract

To reliably predict the impact of climate change on ecosystems, the capacity of organisms to adapt to rapid change needs to be understood. In the context of global warming, temperature is one of the most relevant climate drivers to affect marine ectotherms. Due to fast transmission between generations, transgenerational acclimation may provide a buffer against rapid environmental change such as temperature and provide time for genetic evolution to catch up; thus enabling populations to moderate stressors in the short to medium term. Within this context, we explore for the first time how non-genetic parental effects modify offspring response across three generations of asexually reproducing jellyfish polyps. No parental effects of temperature were observed in the first two generations of polyps (G0 and G1) and polyp reproductive output was driven by the current incubation temperature. In G0, most offspring were produced at 17 °C, and in G1, offspring were produced more rapidly at higher temperatures. In G2, despite only current temperature affecting reproductive timing, average reproductive output was affected by both parental and grandparental temperature, as well as polyps' current incubation temperature. The transgenerational effects of temperature were subtle and appeared most strongly in cooling scenarios, wherein polyps that experienced rapid cooling between generations displayed an immediate drop in reproductive output as opposed to polyps that remained at the same temperature as their parents. Such investigations into transgenerational plasticity highlight the need to examine each population at different temporal scales and within the context of its environment.

4.2 Introduction

Rising air and ocean temperatures are two indicators of global climate change currently taking place (IPCC, 2019). This warming has profound effects on most ecosystems and the animals living within them: increasing the frequency of marine heatwaves (IPCC, 2019; Shanks et al., 2019);

encouraging ocean acidification (Byrne et al., 2010; Klein et al., 2014; Klein et al., 2017); and forcing range shifts (Poloczanska et al., 2013). In marine ecosystems around the world, several species of scyphozoan jellyfish form large blooms in coastal regions, often interfering with human industry and recreation (Kingsford et al., 2018; Dong et al., 2010). These transient blooms typically show large inter-annual variations in both abundance and phenology (Schnedler-Meyer et al., 2018a; Condon et al., 2012), although due to the scarcity of reliable baseline data and a complex life cycle it is unclear how continued changes to the ocean system will affect scyphozoan populations in the future.

Previously believed to be increasing globally (Brodeur et al., 2002; Richardson et al., 2009), further investigation of jellyfish life histories has revealed natural cycles spanning multiple spatial and temporal levels (Brodeur et al., 2008 2013; Gibbons and Richardson, 2013; Schnedler-Meyer et al., 2018a). Scyphozoan jellyfish such as the frequently studied *Aurelia* spp. have life cycles composed of both a mobile, pelagic medusa and a sedentary, benthic polyp (Lucas et al., 2012). This flexible life history enables them to take advantage of rapidly changing environments, as well as having the potential to be a successful invasive species if translocated (Bayha and Graham, 2014). Until recently, polyps passed largely unnoticed due to their smaller visible impact on human activities, unlike their larger medusae counterparts (Purcell et al., 2013; Brotz et al., 2017). However they are now known to be one of the key determinants of the longevity, magnitude and timing of scyphozoan blooms, by maintaining the benthic population across the year and producing ephyrae under appropriate environmental conditions (Lucas et al., 2012; Schnedler-Meyer et al., 2018b).

As a cosmopolitan species containing multiple different lineages and cryptic sister species (Dawson and Jacobs, 2001), how *Aurelia* spp. populations respond to environmental stimuli and the effects of climate change will vary depending on the population and life stage in question (Hubot et al., 2017; Gambill and Peck, 2014). Despite this, many populations are likely to be vulnerable to continued ocean warming (Loveridge et al., 2021). As sedentary benthic organisms, polyp health and reproductive rates are largely determined by *in situ* environmental conditions, with temperature as a key variable dictating growth, survival (Chi et al., 2019), and reproduction (Lucas, 2001; Willcox et al., 2007; Pascual et al., 2015). The thermal optima reflects the seasonal temperature range experienced by population (Riascos et al., 2013) and temperatures beyond these limits will negatively affect survivorship and growth (Purcell et al., 2012; Hubot et al., 2017; Gambill and Peck, 2014). In the context of global warming, it is crucial to understand how populations will respond to future change in their environment. Despite increased focus on the mechanisms and responses to influencing factors that determine reproductive rates (Treible and Condon, 2019; Goldstein and Steiner, 2020), the precise causes of inter-annual and inter-

population variation remain elusive and how populations will respond to future climate change or translocation to novel areas still needs to be investigated (Condon et al., 2012).

The conditions experienced by a polyp may partly determine the performance of its offspring via or epigenetic processes (Lu et al., 2020; Klein et al., 2017). To date, despite careful study of polyps and their role in perpetuating jellyfish populations, very few studies distinguish between different polyp generations (Lu et al., 2020). Transgenerational acclimation encompass previous generations' influences on offspring phenotype (Mousseau and Fox, 1998; Zhang et al., 2006; Youngson and Whitelaw, 2008; Wolf and Wade, 2009), resulting in the phenotypic modification of physiological and behavioural offspring traits by the parent in response to a permanent or temporary stimulus (Youngson and Whitelaw, 2008; Mousseau and Fox, 1998). The extent to which a stressor modifies an offspring phenotype depends on the amplitude, predictability and length of the fluctuation (Mousseau and Fox, 1998; Klein et al., 2017). These effects play a critical role in moderating the response of individuals to stressors such as temperature, acting as a buffer against rapid environmental change, providing time for adaptation and genetic evolution to catch up (Tucić and Avramov, 1996; Marshall et al., 2010; Mousseau and Fox, 1998). The term 'transgenerational plasticity' is generally reserved for sexual reproduction, although there is mounting evidence for similar processes occurring across asexual generations. Similar to Lu et al. (2020), here we define a generation by individuals, i.e. an individual bud is considered the offspring of the individual parent from which it was produced.

In scyphozoan jellyfish, we are only starting to identify how much of polyp response to environmental change is driven by the *in situ* external environment or by offspring internal phenotype (Klein et al., 2017; Lu et al., 2020). The molecular mechanisms behind observed responses are not fully understood, despite past investigations into jellyfish evolution and phylogenetic DNA studies (Dawson; 2001; Fuchs et al, 2014). Molecular biomarkers and heat shock proteins (hsp) have been identified in *Aurelia* spp. (Schroth et al., 2005), but the bulk of jellyfish molecular biology literature lags behind many other fields (Bellantuono et al., 2012; Eirin-Lopez and Putnam, 2018; Franzellitti et al., 2018). Transgenerational effects have been identified in several species across multiple taxa such as the waterflea *Daphnia* sp. and a number of fish species (Veilleux et al., 2015; Wolf and Wade, 2009; Donelson et al., 2016). These remain largely unexplored in scyphozoan jellyfish, where, because of the exclusive asexual reproduction in polyps, these effects may be important in governing population acclimation to changing environmental conditions.

In this study we aimed to characterise the transgenerational effects of a range of realistic summer temperatures on polyps of the scyphozoan *Aurelia aurita* collected from UK temperate coastal

waters. In particular, we aimed to elucidate how much of polyp response is driven by *in situ* conditions and how much is due to offspring phenotype in the scenario of future climate change and warming ocean temperatures. We tested the following hypotheses:

(i) The exposure of parent polyps to different summer temperatures affects (a) the number of offspring produced per polyp; (b) offspring reproductive mode; and (c) reproductive timing.

(ii) In the scenario of warming summer temperatures, offspring of polyps incubated at the same temperature across multiple generations will differ in terms of (a) the number of offspring produced per polyp; (b) offspring reproductive mode; and (c) reproductive timing, than offspring of polyps that experienced increasing temperatures across multiple generations.

In addition, we provide details of an attempt to elucidate the molecular mechanisms behind the observed response by examining the expression of a proxy heat shock protein (HSP70), so that future research can build on our work.

4.3 Methods

4.3.1 Medusa collection and establishment of polyp cultures

Polyps of *Aurelia aurita* (Dawson et al., 2015) were settled from planula larvae taken from mature female medusae collected on 18th January 2018 from Horsea Lake, UK (50°49’58.8; -1°05’36.9), when the ambient surface temperature was 6.3 °C and salinity was 21.9. Horsea Lake is a brackish, semi-enclosed, man-made body of water connected to Portsmouth Harbour via a controlled pipe and valve, and the bottom (6 m) water temperature typically ranges from 5.5 °C in February to 23.0 °C in July, remaining below 10°C from November to March (Lucas, 1996; CEFAS, 2018).

Medusae were maintained in a kreisel at the National Oceanography Centre Southampton aquarium. Fully developed polyps that settled on the glass surface were removed using a scalpel and Pasteur pipette and 90 were reattached in individual 60 ml clear polystyrene pots.

4.3.2 Polyp maintenance

Generation 0 (G0) replicates were maintained in individual 60 ml microcosms at 15 °C for 14 days before the start of the experiment. Only G0 polyps that reattached were used in the experiment. Over 21 days, thirty replicates per group were either maintained at, or, transitioned to experimental temperatures (15 °C, 17 °C, 19 °C). All replicates were maintained at their

experimental temperatures for a week before the start of the experiment. Any offspring produced by asexual reproduction before the start of the experiment were removed.

Food was supplied at non-limiting quantities 3 days a week, directly on to polyp tentacles using a Pasteur pipette to minimise uneaten food remaining in the water. Due to suggestions that *Artemia* nauplii are not of sufficient quality to sustain polyps (Lesniowski et al., 2015), a combination of ZM100 (80-200 µm dried zooplankton) mixed with 1 day-old *Artemia* nauplii were fed to the polyps. Salinity was maintained at 31 following a week-long transition from the salinity at collection. Seawater was sourced from Southampton Water and passed through pressurised sand filters, a UV steriliser, a protein skimmer and a de-nitrifier before use. When required, reduced salinity water was created by adding reverse osmosis water to seawater until the desired salinity was achieved. Polyp cultures were maintained in darkened temperature-controlled incubators apart from when measurements were being taken (<1 minute) or when being fed (<1 minute) to minimise algal growth and to remove any confounding effects of the dark/light cycle on asexual reproduction (Holst and Jarms, 2007; Liu et al., 2009).

4.3.3 **Experimental set up**

Three successive polyp generations (G0, G1, G2) were incubated at three temperatures representing the range of temperatures encountered in summertime in Southampton Water (Figure 4.1, 15 °C = cold summer; 17 °C = typical summer; 19 °C = warm summer) (Lucas and Lawes, 1998; Lucas et al., 1997). Temperature cycles in Horsea Lake and Southampton Water are very similar (Lucas et al., 1997), and temperature data from Southampton Water were used because it has longer data records (1984–2012) revealing more of the inter-annual variability (CEFAS, 2018). Successive generations were moved to different temperature conditions in the order outlined in Figure 4.1. G1 replicates, budded offspring of G0 replicates, were gently removed from parent polyps using a pipette and placed in an individual 60mL clear polystyrene pot. G2 replicates were the budded offspring of G1 replicates, and moved in the same way as G1 replicates. Each experiment lasted for 49 days from the introduction of the polyp into its individual microcosm, with each replicate followed individually.

Reproductive output (number of stolonal, directly budded polyps and podocysts) and health indicators (colour (scale 1-4), tentacle width and length (thin/fat, long/short), as well as tentacle behaviour (retracted/extended), and whether or not the polyp was attached, were recorded on a weekly basis.

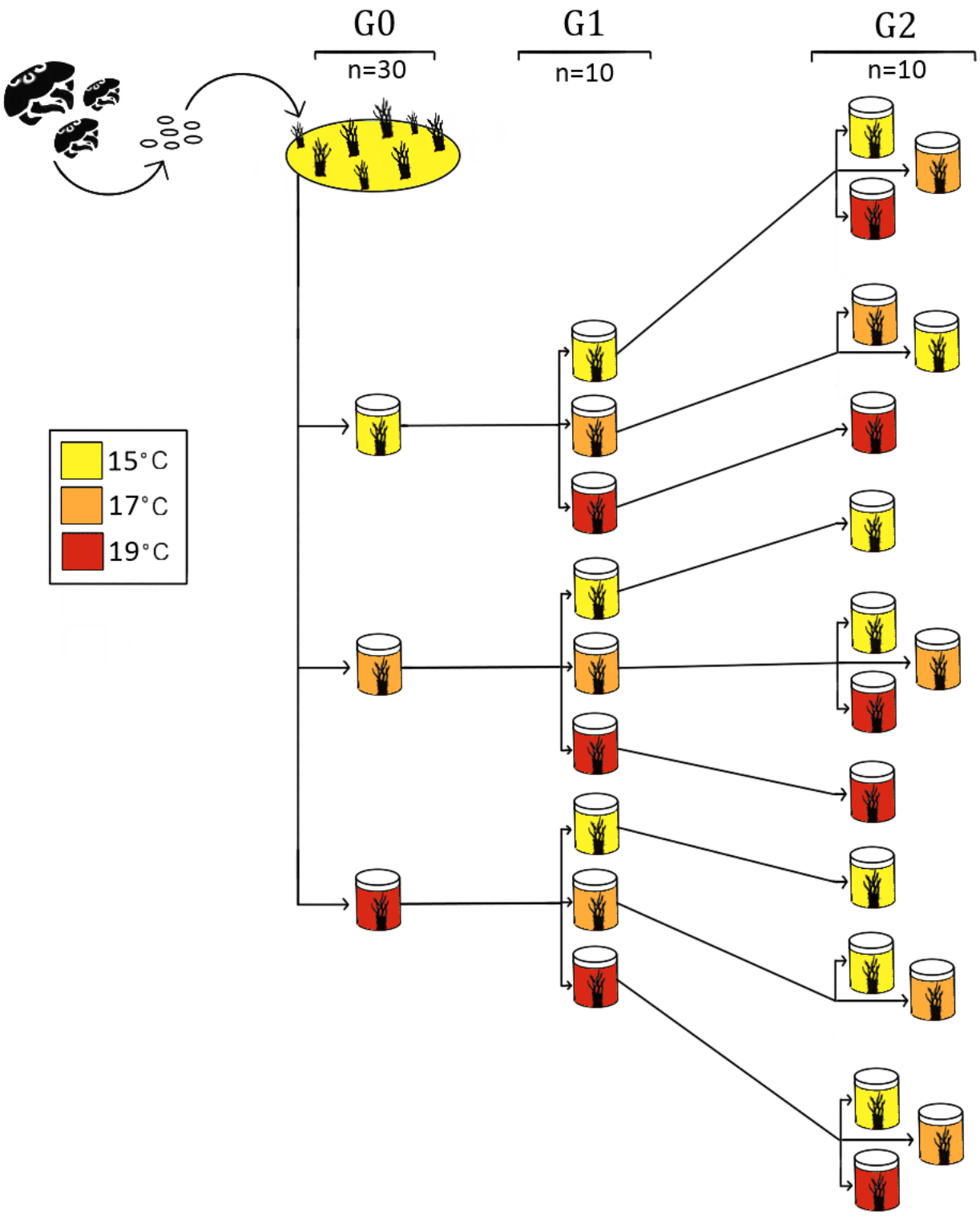

Figure 4.1. Experimental design

4.3.4 **Molecular Analysis**

We attempted to measure the expression of a proxy heat shock protein (HSP70), but this was unsuccessful. Nevertheless, details of the methods can be found in Appendix B.2.

4.3.5 **Data Analysis**

All data analysis was carried out in R V4.0.3. All data were analysed for normality using Shapiro-Wilk tests and QQ plots, and non-parametric tests were carried out if transformation was not sufficient.

Table 4.1. Hypotheses and associated statistical tests

Hypothesis	**Statistical Test**
GO average reproductive output (all modes) ~ current temperature	One-way ANOVA, post hoc Tukey test
G0 average reproductive output (by mode: directly; stolonally budded polyp; podocyst) ~ current temperature	One-way ANOVAs post hoc Tukey test for stolonally budded polyps only
G1 average reproductive output (total output) ~ parental temperature + current temperature	Negative binomial regression model, post hoc Tukey contrasts
G1 average reproductive output (by mode: directly; stolonally budded polyp) ~ parental temperature + current temperature	Negative binomial regression models, post hoc Tukey contrasts
G1 nbr. of days taken to produce offspring ~ parental temperature + current temperature	Negative binomial regression model, post hoc Tukey contrasts
G2 average reproductive output (total output) ~ grandparental temperature * parental temperature * current temperature	Negative binomial regression model
Subsetted by grandparental temperature (17; 19) G2 average reproductive output (total output) ~ parental temperature * current temperature	Negative binomial regression models
Subsetted by grandparental temperature (15) G2 average reproductive output (total output) ~ parental temperature + current temperature	Negative binomial regression model
G2 average reproductive output (by mode: directly; stolonally budded polyp) ~ grandparental temperature * parental temperature + current temperature	Negative binomial regression models
Subsetted by grandparental temperature (19) G2 average reproductive output (by mode: directly; stolonally budded polyp) ~ parental temperature * current temperature	Negative binomial regression model
Subsetted by grandparental temperature (17) G2 average reproductive output (by mode: directly	Negative binomial regression model

Hypothesis	Statistical Test
budded polyp) ~ parental temperature * current temperature	
Subsetted by grandparental temperature (17) G2 average reproductive output (by mode: directly budded polyp) ~ parental temperature + current temperature	Negative binomial regression model
Subsetted by grandparental temperature (15) G2 average reproductive output (by mode: directly; stolonally budded polyp) ~ parental temperature + current temperature	Negative binomial regression model
G2 nbr. of days taken to produce offspring ~ grandparental temperature + parental temperature + current temperature	Negative binomial regression model

4.4 Results

4.4.1 Generation 0 (G0)

In the G0 generation, ≥95% of polyps survived to the end of the experiment in each of the three temperature treatments. Asexual reproduction via the production of direct and stolonal buds, as well as the production of podocysts occurred in all three temperature treatments. No strobilation was observed in any treatment. Temperature had a significant effect on the number of total offspring produced by G0 ($F_{(2, 54)}$=7.906, p<0.001), with G0 polyps incubated at 15 °C producing significantly fewer buds than those incubated at 17 and 19 °C (p<0.001,Figure 4.2).

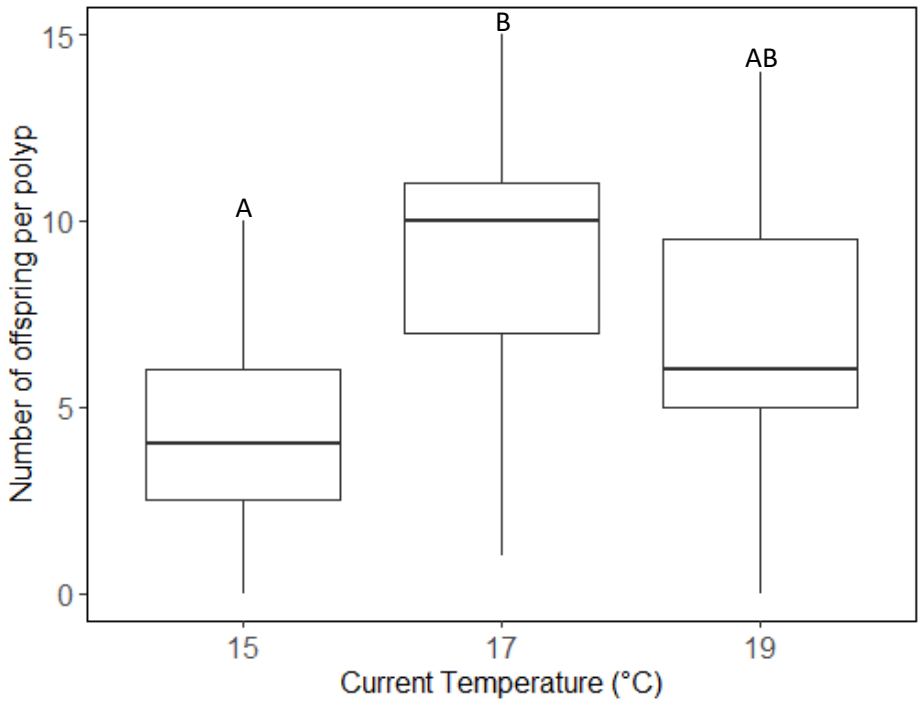

Figure 4.2. Number of offspring produced across the three G0 temperature treatments. A bold line inside each box and whisker plot marks the median. The extremities of the box denote the first and the third quartile and solid vertical lines denote the range. Letters above data points indicate similarities (e.g. A, A), and differences (e.g. A, B) between treatments at single time points, as determined by post hoc tests

There were no significant differences in the number of podocysts or directly budded polyps produced across the three temperature treatments, however temperature significantly influenced the number of stolonally budded polyps produced ($F_{(2, 54)}$=14.47, p<0.001), with polyps incubated at 17 °C produced significantly more stolonally budded polyps than those at 15 °C or 19 °C (p<0.001).

4.4.2 **Generation 1 (G1)**

Average reproductive output (total output)

More offspring were produced by polyps incubated at higher incubation temperatures, no matter the temperature at which their parents were incubated (Z=8.55, p<0.001; Figure 4.3). On average 2.5 additional offspring were produced for every 2 °C increase in replicate incubation temperature.

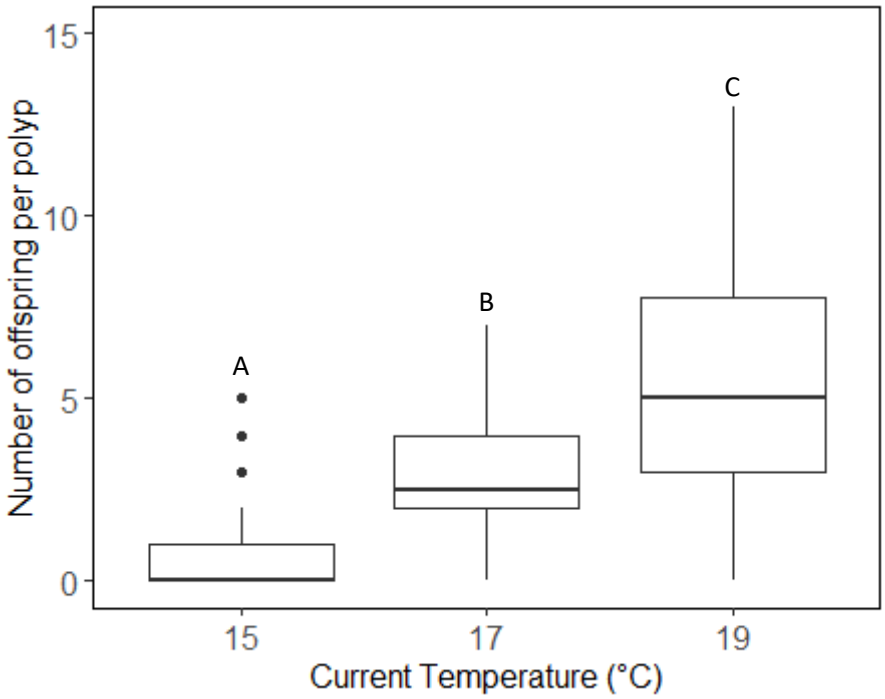

Figure 4.3. Number of offspring produced across the three G1 temperature treatments. A bold line inside each box and whisker plot marks the median. The extremities of the box denote the first and the third quartile and the solid vertical lines denote the range. Any points beyond the solid vertical line are outliers. Letters above data points indicate similarities (e.g. A, A), and differences (e.g. A, B) between treatments at single time points, as determined by post hoc tests

Average reproductive Output (by mode)

There were no significant transgenerational effects of temperature affecting the production of different reproductive modes in G1 polyps. Only their current incubation temperature affected the number of direct buds ($Z=8.287$, $p<0.001$) and stolonal buds ($Z=3.608$, $p<0.001$), with polyps incubated at warmer temperatures producing more of each type no matter their parental temperature ($p<0.001$, Figure 4.4). Podocyst production was insufficient to carry out any robust statistical analysis.

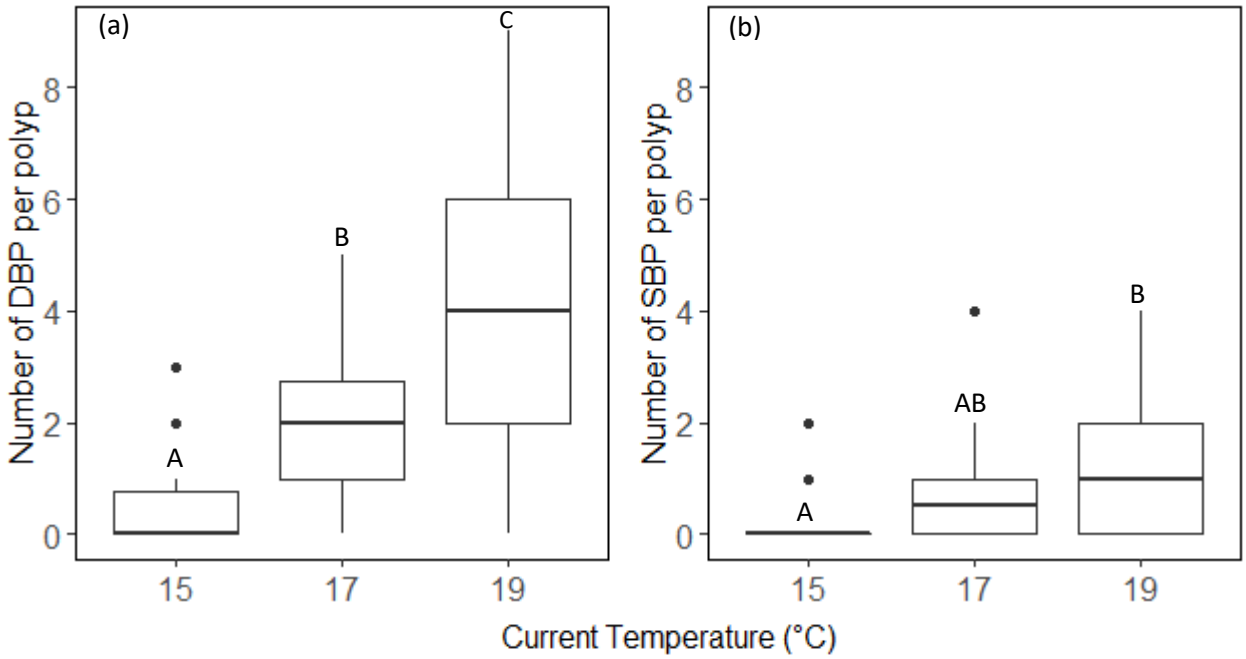

Figure 4.4. Number of (a) directly budded polyps (DBP) and (b) stolonally budded polyps (SBP) produced across the three G1 temperature treatments. A bold line inside each box and whisker plot marks the median. The extremities of the box denote the first and the third quartile and the solid vertical lines denote the range. Any points beyond the solid vertical lines are outliers. Letters above data points indicate similarities (e.g. A, A), and differences (e.g. A, B) between treatments at single time points, as determined by post hoc tests

Reproductive timing

There were no significant transgenerational effects of temperature affecting the number of days to first bud in G1 polyps. Polyps incubated at 17 °C and 19 °C produced their first offspring more rapidly than those incubated at 19 °C temperature, no matter their parental temperature ($Z = -5.648$, $p<0.001$, Figure 4.5). Out of the G1 polyps incubated at 15 °C, 19 polyps did not produce any buds across the experimental period.

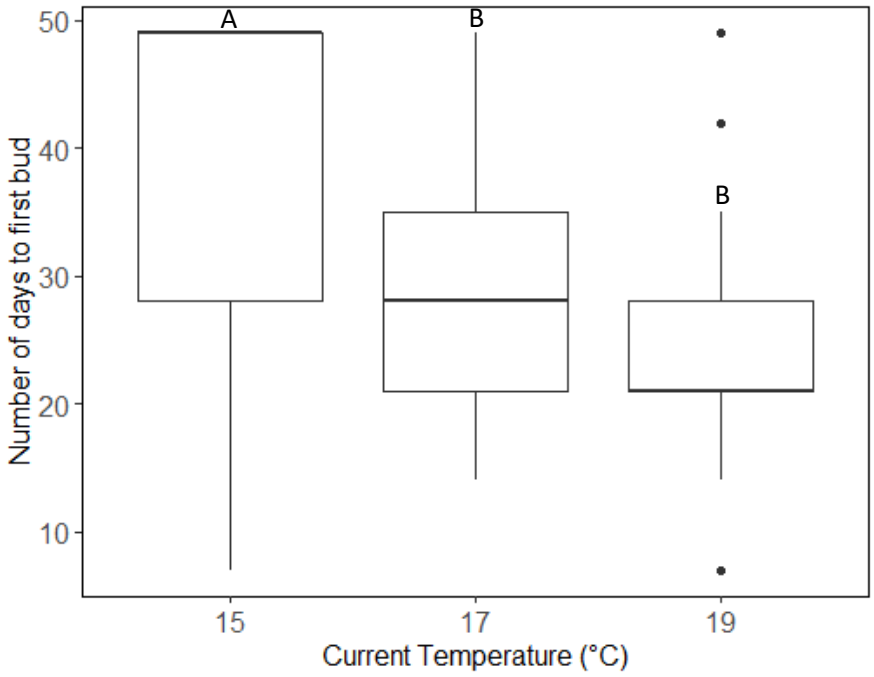

Figure 4.5. Number of days taken for G1 polyps to produce offspring by temperature. A bold line inside each box and whisker plot marks the median. The extremities of the box denote the first and the third quartile and the solid vertical lines denote the range. Any points beyond the solid vertical lines are outliers. Letters above data points indicate similarities (e.g. A, A), and differences (e.g. A, B) between treatments at single time points, as determined by post hoc tests

4.4.3 Transgenerational effects in Generation 2 (G2)

In G2, ≥95% of polyps survived to the end of the experiment in each temperature treatment.

Average reproductive output (total output)

The effect of parental (i.e., G1) temperature on a replicate's offspring production (i.e., G2) was significant, but its effect depended on the replicate's current incubation temperature. As well as this, the interaction between parental and current temperature differed depending on the replicate's grandparental (i.e., G0) temperature ($Z=3.416$, $p<0.001$, Figure 4.6).

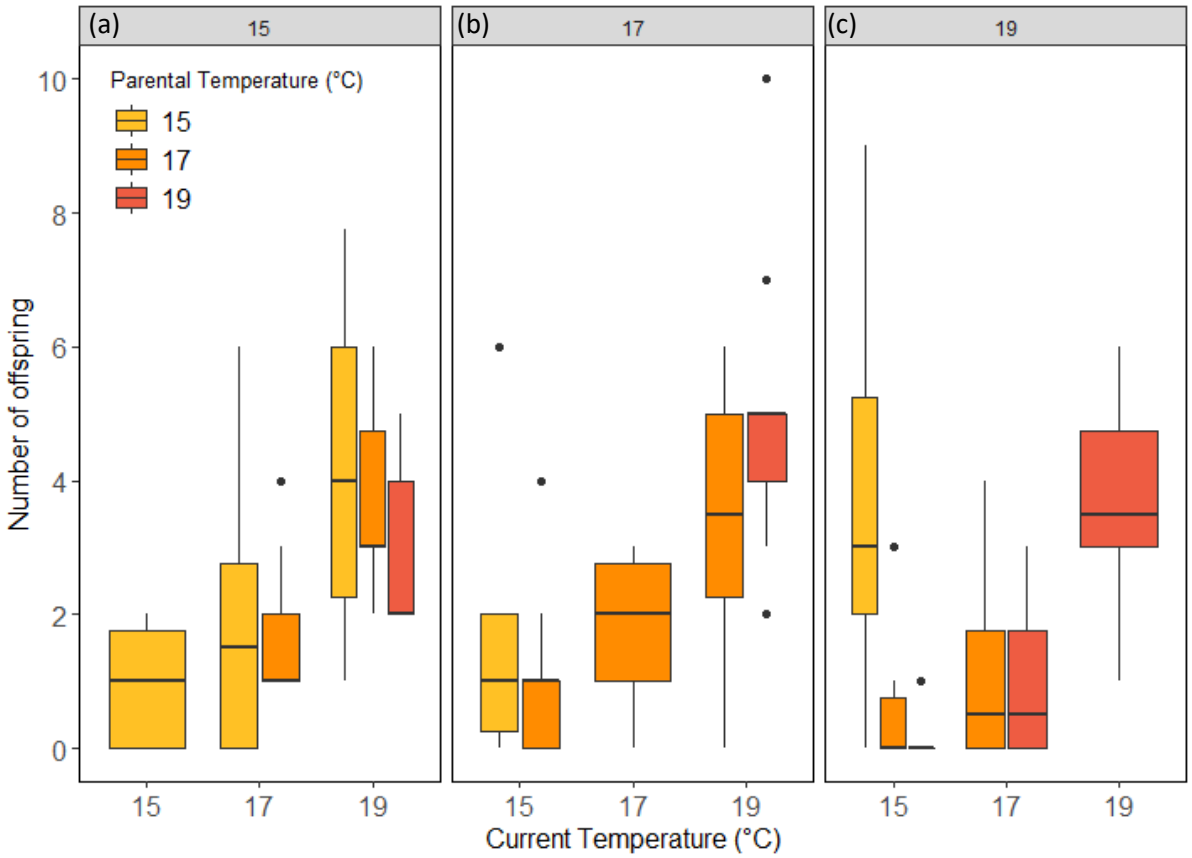

Figure 4.6. Number of offspring produced across the G2 temperature treatments, split according to grandparental (G0) temperature, i.e. a) G0 temperature 15 °C, b) 17 °C, and c) 19 °C. Colour indicates parental (G1) temperature. A bold line inside each box and whisker plot marks the median. The extremities of the box denote the first and the third quartile and the solid vertical lines denote the range. Any points beyond the solid vertical lines are outliers

Amongst polyps whose grandparents were incubated at 15 °C, there was no significant interactive effect between parental and current incubation temperature influencing offspring production ($p<0.05$, Figure 4.6a). Current temperature had a significant effect on offspring production, with more offspring being produced at higher temperatures ($Z=2.523$, $p<0.05$).

Amongst polyps whose grandparents were incubated at 17 °C, offspring production was influenced by parental incubation temperature, but the strength of this effect depended on the polyp's current temperature ($Z=2.523$, $p<0.05$, Figure 4.6b).

Finally, there was a strong effect of parental temperature on polyps whose grandparents were incubated at 19 °C, and this effect also depended on the current incubation temperature of the

polyp (Z=3.350, p<0.001, Figure 4.6c). G2 polyps currently incubated at 15 °C produced over twice as many offspring when their parent was also incubated at 15 °C, as opposed to 17 or 19 °C.

Average reproductive output (by mode)

The effect of parental temperature on polyps' directly budded polyp and stolonally budded polyp production was significant, but the effect depended on the replicate's current incubation temperature. As well as this, the interaction between parental and current incubation temperature differed depending on the replicate's grandparental temperature for directly budded polyps (Z=2.768, p<0.01) and stolonally budded polyps (Z=2.538, p<0.05).

Amongst replicates whose grandparents were incubated at 15 °C, only current temperature significantly influenced the production of directly budded polyps (Z=3.462, p<0.001) and stolonal budded polyps (Z=3.736, p<0.001), with more direct buds and stolonal buds produced at current warmer temperatures.

Amongst replicates whose grandparents were incubated at 17 °C, directly budded polyp production was affected by parental incubation temperature, but the strength of this effect depended on the replicate's current temperature (Z=2.151, p<0.05, Figure 4.7). Stolonal bud production was only affected by polyps' current incubation temperature (Z=2.151, p<0.05).

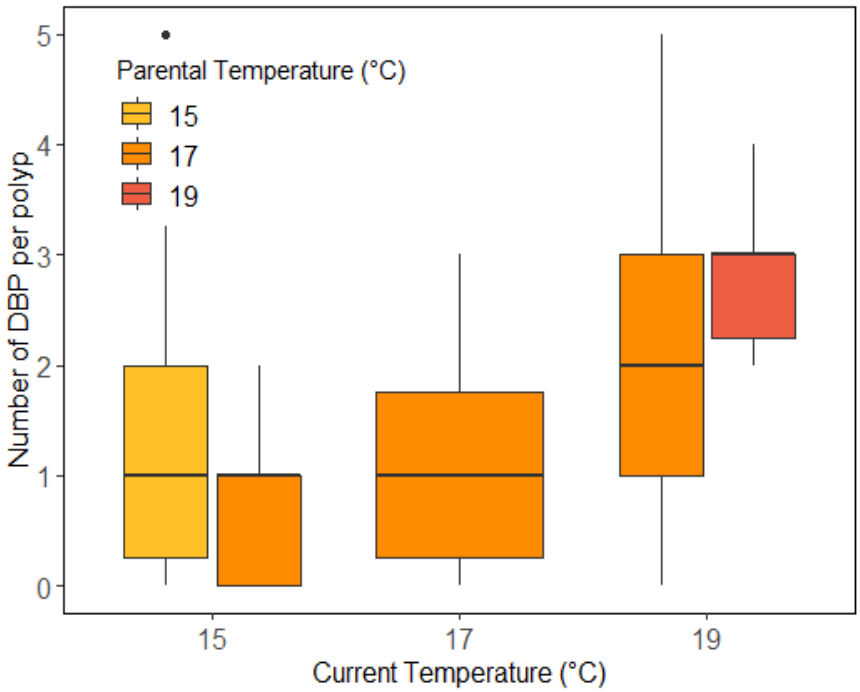

Figure 4.7. Number of directly budded polyps produced by polyps whose grandparents were incubated at 17 °C. A bold line inside each box and whisker plot marks the median. The extremities of the box denote the first and the third quartile and the solid vertical lines denote the range. Any points beyond the solid vertical lines are outliers

Amongst replicates whose grandparents were incubated at 19 °C, directly budded polyp production was affected by parental incubation temperature, but the strength of this effect depended on the replicate's current temperature (Z=2.422, p<0.05,Figure 4.8). Podocyst production was insufficient to carry out any robust statistical analysis.

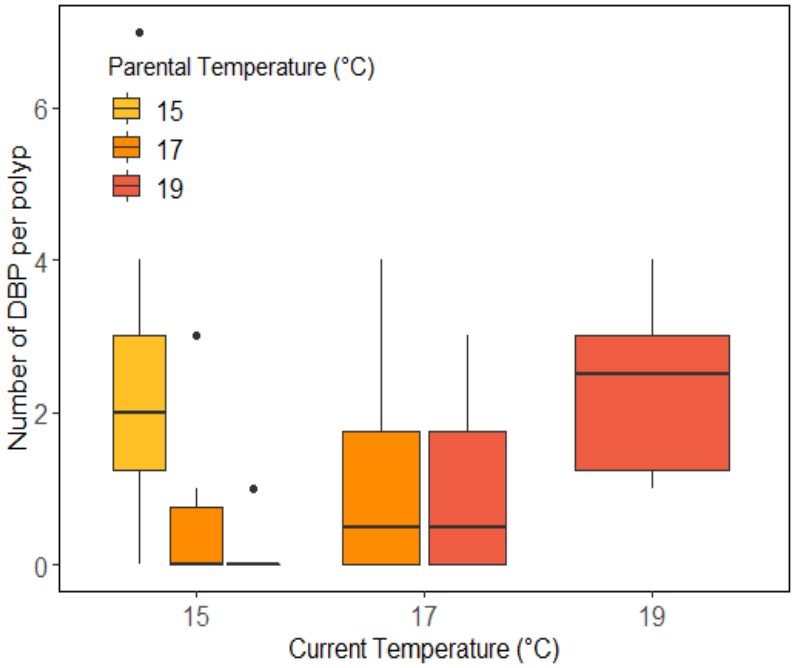

Figure 4.8. Number of directly budded polyps produced by polyps whose grandparents were incubated at 19 °C. A bold line inside each box and whisker plot marks the median. The extremities of the box denote the first and the third quartile and the solid vertical lines denote the range. Any points beyond the solid vertical lines are outliers

Reproductive timing

Day to first bud in G2 significantly decreased with increasing current incubation temperature (Z=-6.128, p<0.001, Figure 4.9) and there were no observed transgenerational effects of temperature on reproductive timing. At 19 °C, G2 polyps took on average 23 days to produce their first bud, as opposed to 30 days at 17 °C and approximately 49 days at 15 °C degrees.

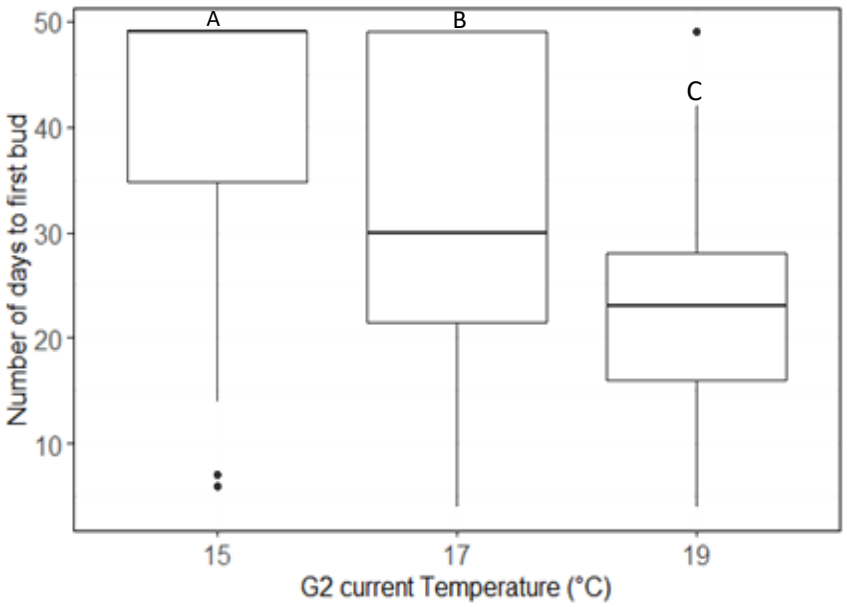

Figure 4.9. Number of days taken for G2 polyps to produce offspring by temperature group. A bold line inside each box and whisker plot marks the median. The extremities of the box denote the first and the third quartile and the solid vertical lines denote the range. Any points beyond the solid vertical lines are outliers. Letters above data points indicate similarities (e.g. A, A), and differences (e.g. A, B) between treatments at single time points, as determined by post hoc tests

4.5 Discussion

Across the 4 °C temperature range used in this experiment, current temperature was the driving variable behind polyp reproductive activity, influencing both total number of offspring produced as well as the number of days taken to produce their first offspring. Thermal regimes are a key determinant of polyp's reproductive output: thermal windows are as narrow as possible to minimise maintenance costs and increase efficiency, with steep declines at temperatures beyond thermal limits (Höhn et al., 2017). This could explain why the temperate scyphozoan *Aurelia aurita* is known to have a wide tolerance to environmental conditions as it is well adapted to UK coastal waters that undergo large seasonal variations in temperature from 6 °C to 23 °C. In our experiment, G1 and G2 polyps produced more offspring at warmer temperatures, indicating that our highest experimental temperature (19 °C) was within this population's thermal limits. As well as this, the number of days taken by polyps to produce their first offspring was less at warmer temperatures. Producing increased numbers of offspring more rapidly at warmer summer temperatures will prove advantageous to polyp colonies, both in maintaining the benthic

population across the year, as well as when producing ephyrae following suitable environmental cues (Han and Uye, 2010). Provided this reproductive growth is sustained with sufficient food resources, increased reproductive output may compensate for mortality caused by predation, inter- and intra-specific competition for space and food and physiological stress experienced across the year (Lucas et al., 2012).

Transgenerational effects of temperature have been observed in a range of taxa (Burgess and Marshall, 2011; Donelson et al., 2012; Groot et al., 2017). In parthenogenetic *Daphnia magna* females, maternal incubation temperature affects offspring resistance to bacterial parasites and other pathogens (Garbutt et al., 2014), as well as increasing resistance to toxic cyanobacteria at high temperatures (Lyu et al., 2017). In the coral *Acropora tenuis* parental effects account for variations in larval settlement success and associated *Symbiodinium* communities (Quigley et al., 2016). Effects are most likely to be environmentally adaptive if the offspring are faced with the same stressors and environment as the previous generation (Salinas and Munch, 2012; Mousseau and Fox, 2014). For example, a strong adaptive response was observed in Sheepshead minnows where offspring from high and low temperature parents grew best at their parental temperatures, provided there was sufficient exposure time to this temperature (Mousseau and Fox, 1998; Salinas and Munch, 2012). Similarly, Putnam and Gates (2015) observed that when adults of the reef building coral *Pocillopora damicornis* were exposed to warmer, more acidic conditions, larvae were able to better respond to similar post-release conditions.

We examined three scenarios across three subsequent generations of asexual polyps: transgenerational warming, transgenerational cooling, and constant temperature. Replicates that remained at the same temperature as their parent produced more offspring than those that experienced transgenerational cooling across a generation. This could indicate that within the 4 °C range examined in our experiment, transgenerational effects of temperature could offer an advantage to polyps that experience the same conditions as their parents. However, in regions with large seasonal variations in temperature, such as in UK coastal waters, these effects may not prove beneficial. Similar to Lu et al. (2020), we observed significant inhibition of budding activity in cooling scenarios. In the winter and at colder experimental temperatures, bud production slows and can reduce to almost zero (Willcox et al., 2007). Seasonal cooling in the autumn and winter may therefore dampen polyp colony growth in the winter period due to a mismatch between parental and offspring thermal conditions.

Global climate change poses significant threats to marine organisms, through changes to phenology (Parmesan, 2007) and range (Zhang et al., 2020). For example, in response to fluctuations in the hydroclimatic environment *Calanus* spp. copepods have shifted their range

further north in the NE Atlantic (Beaugrand, 2003). For species dependent on spring plankton blooms, these changes could potentially cause a large disruption to reproduction and growth. As well as this, with some regions experiencing rapid warming (Belkin, 2009; IPCC, 2019), populations are likely to be vulnerable to continued warming beyond their thermal limits (Höhn et al., 2017). Thermal transgenerational plasticity may provide a buffer against the effects of climate warming (Bellantuono et al., 2012). However, it is still unclear how evolution, phenotypic plasticity and transgenerational plasticity interact to generate long-term responses (Salinas and Munch, 2012). For example, in the tropical Irukandji jellyfish *Alatina alata*, pre-exposure of parent polyps to elevated temperatures and reduced pH did not confer any advantage on to offspring polyps and both parent and offspring polyps responded similarly to the treatments (Klein et al., 2017).

In our experiment we did not uncover any effects of transgenerational warming increasing the fitness or resilience of offspring in the face of continued climate warming. Parental temperature did not affect G1 reproductive output and in G2 reproductive timing was only driven by current incubation temperature. Polyps who experienced transgenerational warming across subsequent generations did not produce significantly different numbers of offspring than polyps who were maintained at constant warm temperatures across multiple generations. Our results indicate that, at our temperature range of 15-19 ^{o}C, transgenerational effects did not significantly benefit polyp reproductive output. The current environmental temperature that polyps experience is likely to play a much larger role in determining reproductive output. If temperatures increase past population thermal limits, which did not happen in this experiment, polyps are likely to experience a sharp decline in reproductive activity (Höhn et al., 2017). Consequently, determining the thermal limits of different populations with the aim of highlighting potentially vulnerable regions is critical to understanding how continued ocean warming will affect scyphozoan jellyfish populations.

The extent to which a stressor modifies an offspring phenotype depends on the amplitude, predictability, and length of the fluctuation (Klein et al., 2017; Mousseau and Fox, 1998). As such, how an effect is expressed, either visibly in an organism's reproductive output, or in the offspring's phenotype, will vary depending on the environment and genotype in question. Responses to environmental drivers are therefore highly contextual to the spatio-temporal scale of the stressor (Mousseau and Fox, 2014). Across smaller temperature ranges, such as the 4 °C range examined in this study, transgenerational effects are subtle, and may require an increased number of generations to become fully apparent. For example, in our experiment, only the third generation of polyps (G2) presented visible influences of past generations on offspring production, whereas across the 8 °C range as used by Lu et al. (2020), effects became visible in the second generation of polyps. Examining larger temperature ranges is likely to result in more

visible effects that may become apparent in fewer generations (Lu et al., 2020). Nevertheless, despite observing no visible response in G1, it is likely that phenotypic modification of offspring traits was occurring at the molecular level. For example, Pespeni et al. (2013) examined the response of the purple sea urchin *Strongylocentrotus purpuratus* to elevated CO_2. No visible signs of change were recognised, although changes across hundreds of loci were detected, suggesting that there was potential for change (Pespeni et al., 2013). Consequently, a molecular component to experiments is key to understanding transgenerational effects. Due to contamination issues, our molecular examination of the heat shock protein Hsp70 was not successful; however, we would strongly recommend further investigations into the molecular component of transgenerational plasticity.

Our experiment benefitted from taking place over three clearly defined generations of asexual polyps, providing further insights into the characterisation of transgenerational acclimation in the scyphozoan jellyfish *Aurelia aurita*. Our attempt at molecular characterisation of the Hsp70 gene expression provides insight into the challenge of working with RNA in difficult subjects such as scyphozoan jellyfish polyps, yet provides a foundation for future work in this area. Future investigations should investigate population-specific responses to environmental change using common garden experiments, to understand how transgenerational effects may affect populations in the context of future environmental change.

4.6 **Conclusions**

This study focuses on further characterising the transgenerational effects of a range of realistic summer temperatures on polyps of the scyphozoan *Aurelia aurita* collected from UK temperate coastal waters. Transgenerational effects were subtle and required multiple generations to express visibly in polyps. Effects were most visible in cooling scenarios and could contribute to the decline in reproductive activity in the winter. Finally, despite an unsuccessful attempt to elucidate the molecular mechanisms behind the observed response by examining the expression of a proxy heat shock protein (HSP70), our research provides an avenue for future research efforts in this area.

Chapter 5 Shorter, warmer winters may inhibit production of ephyrae in a population of the moon jellyfish *Aurelia aurita*

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5.1 Abstract

Scyphozoan jellyfish blooms display high interannual variability in terms of timing of appearance and size of the bloom. To understand the causes of this variability, the conditions experienced by the polyps prior to the production of ephyrae in the spring were examined. Polyps reared from planula larvae of *Aurelia aurita* medusae collected from southern England (50°49'58.8; -1°05'36.9) were incubated under orthogonal combinations of temperature (4, 7, 10 °C) and duration (2, 4, 6, 8 weeks), representing the range of winter conditions in that region, before experiencing an increase to 13 °C. Timing and success of strobilation were recorded. No significant production of ephyrae was observed in any of the 2- and 4-week incubations, or in any 10 °C incubation. Time to first ephyra release decreased with longer winter incubations, and more ephyrae were produced following longer and colder winter simulations. This experiment indicates that *A. aurita* requires a minimum period of cooler temperatures to strobilate, and contradicts claims that jellyfish populations will be more prevalent in warming oceans, specifically in the context of warmer winter conditions. Such investigations on population-specific ontogeny highlights the need to examine each life stage separately as well as in the context of its environment.

5.2 Introduction

Scyphozoan medusae are key components of marine ecosystems (Richardson et al., 2009), providing essential ecosystem services (Doyle et al., 2014; Yusuf et al., 2018) despite their frequent portrayal as trophic dead ends, or in the media as dangerous nuisances (Lunney and Moon, 2008). Whilst there is still much debate surrounding the extent of the anthropogenic influence on jellyfish population dynamics (Purcell, 2012; Pitt et al., 2018), if present in large numbers jellyfish blooms can cause problems for human coastal activities (Lynam et al., 2006; Roux et al., 2013; Remigante et al., 2018).

One of the most frequent blooming species along the south coast of England is *Aurelia aurita* (Linnaeus, 1758) (Piksley et al., 2014; Dawson et al., 2015). *Aurelia* is a cosmopolitan genus of Scyphozoa (Russell, 1970) comprising multiple cryptic species living in coastal and shelf sea environments between 70°N and 40°S (Dawson and Jacobs, 2001). Like many scyphozoans, it has a complex life cycle comprising a pelagic sexual medusa and a benthic asexual polyp (Lucas, 2001; Fuchs et al., 2014). Through the production of ephyrae via strobilation, polyps directly and indirectly influence where medusa populations occur, the seasonal and interannual variability in medusa abundance, and may even be key to the success or failure of a jellyfish bloom (Gröndahl, 1988b; Schnedler-Meyer et al., 2018a).

Strobilation in *Aurelia aurita* has been studied in a variety of populations in the laboratory (Holst, 2012; Fuchs et al., 2014; Sukhoputova and Kraus, 2017). In situ, most populations strobilate following the seasonal cooling and subsequent warming of the water (Brewer, 1989; Feng et al., 2018), alongside changes in other variables such as salinity (Holst and Jarms, 2010), light (Custance, 1964; Purcell et al., 2009), oxygen (Condon et al., 2001), tidal rhythms (Calder, 1974) and food supply (Lucas and Williams, 1994). Different polyp populations vary widely in their response to forcing variables and authors have suggested varied and sometimes contradicting triggers (Sukhoptova & Kraus, 2017). For example, in many temperate locations ephyrae are typically released in spring (Lucas, 1996' Schnedler-Meyer et al., 2018), or following ice-out in late February in the Niantic River, USA (Brewer, 1989). However, some populations, such as those in the Suez Canal, strobilate when they reach the winter minimum temperature (Hamed and Khaled, 2011; Sukhoputova and Kraus, 2017). Others follow tidal rather than temperature cues: in Roscoe Bay, Canada, strobilation occurs in June and July coinciding with the lowest summer tides (Albert and Walsh, 2014). Finally, in Horsea Lake, UK, from where the current study animals originate, most *A. aurita* ephyrae appear in early spring around February and March (Lucas, 1996).

Despite the range of responses to a number of forcing variables, temperature consistently appears as one of the main triggers of strobilation, although the magnitude and direction of

temperature change (i.e. cooling vs. warming) is inconsistent between studies (Holst, 2012; Treible and Condon, 2019). Thermal windows constrain polyps' latitudinal distributions, and temperature determines the rate at which physiological processes occur (Gambill and Peck, 2014; Höhn et al., 2017). The gene CL390 is a temperature dependent molecular timer that interacts with the RxR transcription factor to distinguish between short-term temperature fluctuations and seasonal changes, regulating the polyp-to-jellyfish transition in *Aurelia* spp. (Fuchs et al., 2014; Shi et al., 2018). It is produced gradually at low temperatures, initiating metamorphosis once an activation threshold is reached. This results in the initiation of strobilation only after a sufficient period of time at low temperatures (Fuchs et al., 2014). Purcell et al. (2009) showed that 2.9 times more *Aurelia* spp. polyps strobilated when the pre-strobilation temperature increased by less than 1 °C. However, a number of other studies propose that a period at colder temperatures is necessary to ensure that ephyral growth and development occurs in spring, when temperate species can take advantage of the spring bloom as the lowest numbers of ephyrae tended to be produced at the highest temperatures (Lucas, 2001; Widmer et al., 2016).

Alongside increased food availability, warmer winter periods may promote the production of certain populations of temperate scyphozoan medusae such as *Aurelia aurita* and *Cyanea lamarckii* (Purcell et al., 2012, Goldstein & Steiner, 2020). However, large-scale climatic variability has the potential to modify the timing and abundance of phytoplankton blooms, and to directly change zooplankton community structure (Edwards and Richardson, 2004; Hays et al., 2005). In such regions, certain scyphozoan jellyfish, such as *A. aurita*, can act as indicators of ecosystem variability (Lynam et al., 2004). For example, across the North Atlantic, the North Sea and Europe, the North Atlantic Oscillation (NAO) influences the variability of weather systems, both directly and indirectly affecting marine ecosystems (Beaugrand, 2003). The NAO has a strong positive correlation with sea surface temperature in areas such as the North Sea, where it is at its strongest in spring and winter. This period coincides with *A. aurita* ephyra release in early spring (Lucas, 2001, Lynam et al., 2004). A strong inverse relationship exists between the winter NAOI and the median abundance of *A. aurita* medusae in this region, suggesting that especially in the winter and spring months, increased sea surface temperatures during high NAOI phases may contribute to poor strobilation and ephyral development, resulting in smaller populations of medusae in the summer (Lynam et al., 2004). To add to these observations and further elucidate this link between the hydroclimatic environment and *A. aurita* populations, it is necessary to understand to what extent variable winter temperatures influence *A. aurita* polyp reproduction.

Despite being mentioned in Treible & Condon (2019) as a determining factor of the phenology in strobilation, duration of the winter period has not been examined as a factor influencing strobilation in the spring, even though the gene CL390 necessitates a certain period at cooler

temperatures to initiate strobilation (Fuchs et al., 2014). To predict the timing and magnitude of ephyra release, it is essential to understand how the conditions preceding strobilation influences a population, from triggering the strobilation process, to the final number of ephyrae produced. Here we report on a laboratory experiment investigating the effects of different winter temperatures and durations on the production of ephyrae by scyphistomae originating from Horsea Lake in the UK. The following hypotheses were tested: differences in the duration and temperature of the winter period would significantly affect (i) the time from the temperature increase to strobilation, (ii) the proportion of polyps strobilating, and (Bayha et al., 2012) the total numbers of ephyrae produced.

5.3 Methods

5.3.1 Establishment of polyp cultures

Polyps of *Aurelia aurita* were settled from planula larvae taken from three mature female medusae collected from Horsea Lake, UK (50°49'58.8; -1°05'36.9) on 28th June 2018, when the ambient surface temperature was 23 °C and the salinity 23.5 (Dawson et al., 2015). Horsea Lake is a brackish, semi-enclosed, man-made body of water connected to Portsmouth Harbour via a controlled pipe and valve, and the bottom (6 m) water temperature typically ranges from 5.5 °C in February to 23.0 °C in July, remaining below 10 °C from November to March (Lucas, 1996; CEFAS, 2018). The winter minimum usually occurs in February at 7 °C but has ranged from 10 °C in 2008 to 3.7 °C in 1986 (CEFAS, 2018).

Medusae and released larvae that settled into polyps were maintained for 3 months in a kreisel at the National Oceanography Centre Southampton aquarium. Fully developed polyps that settled on the glass surface were removed using a scalpel and Pasteur pipette and reattached by placing polyps directly on the bottom of individual 60 ml clear polystyrene pots filled with water at the same salinity, maintained in darkness. The pots had been preconditioned by filling them with seawater 24 h before the procedure.

5.3.2 Polyp maintenance

One hundred and sixty reattached polyps were maintained in individual 60 ml microcosms at 15 °C for 14 days before the start of the experiment. Any offspring (podocysts, directly and stolonally budded polyps or ephyrae) produced before the start of the experiment were removed using a scalpel and Pasteur pipette after naturally separating from the parent polyp. Over 7 days

(Widmer et al., 2016) replicates were transitioned to experimental temperatures (4 °C, 1.5 °C day^{-1}; 7 °C, 1.1 °C day^{-1}; 10 °C, 0.7 °C day^{-1}; and 13 °C, 0.2 °C day^{-1}).

Salinity was maintained at 23.5 and seawater was completely exchanged once a week. Seawater was sourced from Southampton Water and passed through pressurised sand filters, a UV steriliser, a protein skimmer and a de-nitrifier before use. When required, reduced salinity water was created by adding reverse osmosis water to seawater until the desired salinity was achieved. Food was supplied at non-limiting quantities once a week, directly on to polyp tentacles using a Pasteur pipette to minimise uneaten food remaining in the water. Due to suggestions that *Artemia* nauplii are not of sufficient quality to sustain polyps (Lesniowski et al., 2015), a combination of ZM100 (80-200 micron dried zooplankton) mixed with 1-day-old *Artemia* nauplii were fed to the polyps. Polyp cultures were maintained in darkened temperature-controlled incubators apart from when measurements were being taken (<1 min) or when being fed (<1 min) to minimise algal growth and to remove any confounding effects of the dark/light cycle on asexual reproduction (Holst and Jarms, 2007; Liu et al., 2009). Any offspring (podocysts, directly and stolonally budded polyps or ephyrae) produced during the experiment were recorded and removed using a scalpel and Pasteur pipette after naturally separating from the parent polyp.

5.3.3 **Experimental setup**

The experiment consisted of two orthogonal factors: temperature (three levels: 4, 7 and 10 °C) and duration (four levels: 2, 4, 6, 8 weeks; Figure 5.1). Controls were maintained at a constant temperature (4, 7, 10 or 13 °C) for 12 weeks. Each treatment had 10 replicates, each comprising a single polyp in a 60 ml microcosm. Following each incubation period, all treatments were moved to 13 °C (average springtime temperature) for 4 weeks. If after 4 weeks there was no evidence of strobilation (i.e. lateral constrictions of the polyp, darkened colour, retraction of tentacles or presence of ephyrae) the treatment was terminated. If any polyps were about to strobilate or were still strobilating then they were maintained at 13 °C until they released all ephyrae. Controls were not moved to 13 °C and remained at a constant temperature for 12 weeks to demonstrate that a temperature change is necessary to initiate strobilation. Reproductive output (number of stolonal, directly budded polyps, podocysts, and ephyrae), survival and attachment were recorded weekly.

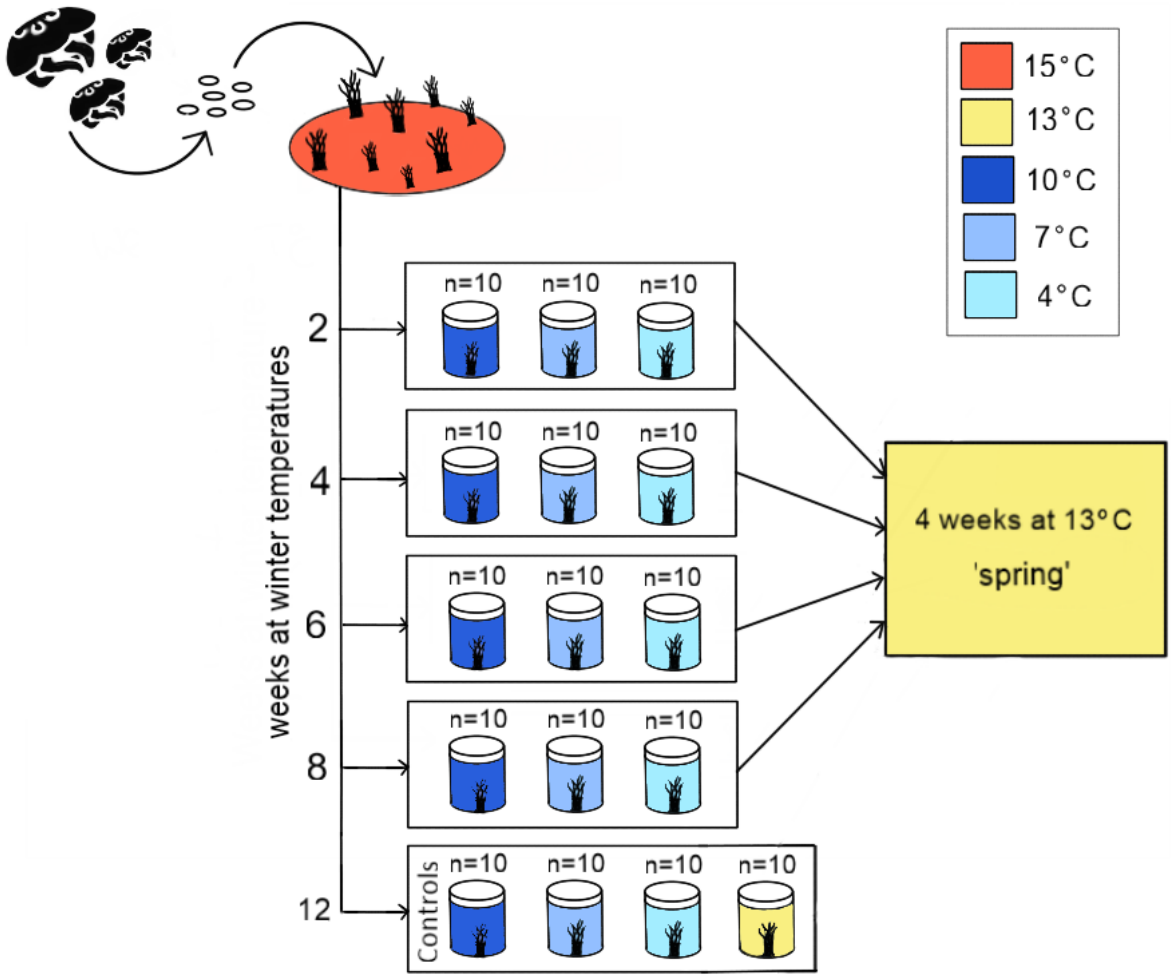

Figure 5.1. Experimental design

Temperature cycles in Horsea Lake and Southampton Water are very similar (Lucas et al., 1997), and temperature data from Southampton Water were used because it has longer data records (1984–2012; Figure 5.2) revealing more of the interannual variability (CEFAS, 2018).

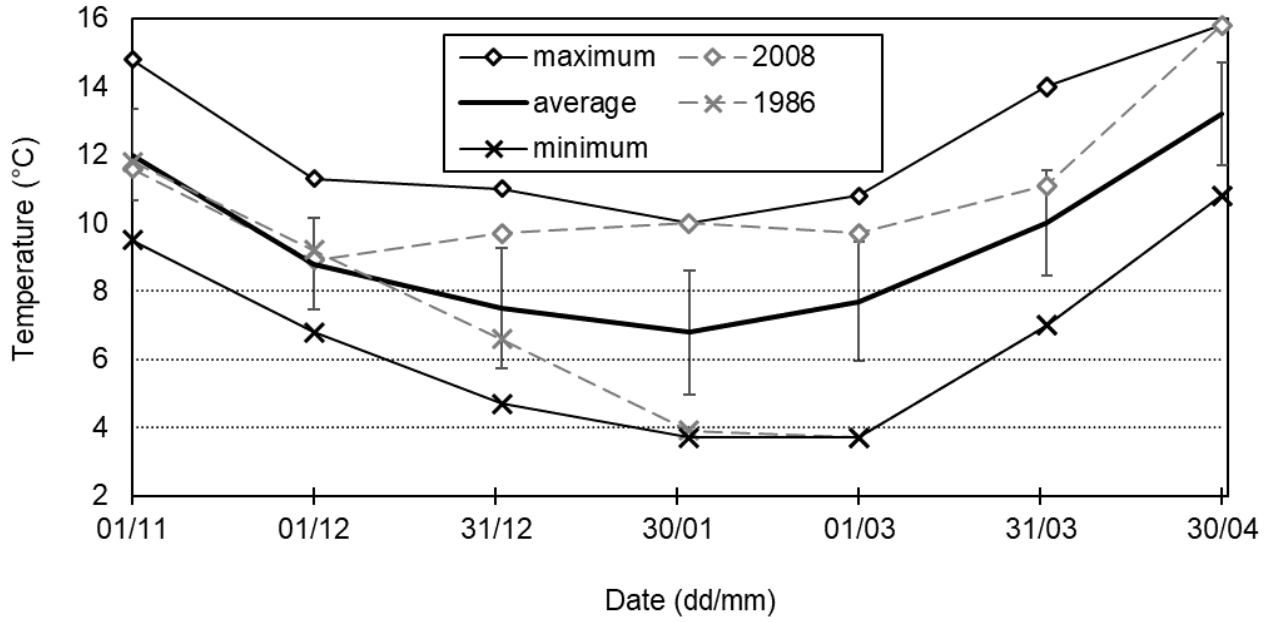

Figure 5.2. Average monthly winter sea surface temperature in Southampton Water from November to May 1984-2012. Data for 1986 and 2008 represent the coldest and warmest years within this dataset. Original data obtained from CEFAS (2018)

5.3.4 **Data analyses**

No ephyrae were produced in the 2-week treatments or the 12-week controls, and only 2 ephyrae were produced in the 4-week treatment. Only replicates that produced at least one ephyra were included in the analyses. Time from the end of the winter simulation to first ephyra release was analysed using a two-way ANOVA. The factors were Duration (two levels; 6 and 8 weeks) and Temperature (three levels: 4, 7 and 10 °C). As no significant interaction occurred between temperature and duration, the interaction term was removed, and an additive analysis was carried out. The number of replicates that strobilated within each treatment was analysed using a logistic regression model (family: binomial; n = 10). As no significant interaction occurred between temperature and duration, the interaction term was removed, and an additive analysis was carried out. Post hoc tests were consequently conducted separately within temperature (4, 7, 10 °C; n = 40/temperature) and duration (2, 4, 6, 8 weeks; n = 30/duration) groups. Finally, since the dataset on number of ephyrae produced was zero inflated, a negative binomial regression model was created to analyse the influence of temperature and duration on the total number of ephyrae produced per replicate (n = 10).

Prior to analysis, data were tested for normality (Shapiro–Wilk test) and homogeneity of variance (Bartlett test). If variances could not be stabilised by transformation, the p-value was reduced to 0.01 to reduce the risk of Type I error. Best fitting models were chosen based on Akaike

Information Criterion (Kogovšek et al., 2018). When significant differences were detected post hoc multiple comparisons of means (Tukey contrasts) determined which treatments differed.

5.4 Results

5.4.1 Survival and budding

All replicates survived to the end of the experiment except for a single replicate in each of the 4 weeks/ 10 °C and 8 weeks/4 °C treatments that died (disintegration of polyp body) before the temperature was increased to 13 °C. Minimal budding and podocyst production was observed in all treatments (See Appendix C for further details).

5.4.2 Days from temperature increase to first ephyrae release

Time to first ephyra release decreased significantly with increasing winter duration, with replicates incubated for 8 weeks at winter temperatures releasing ephyrae on average 10 days earlier than those incubated for 6 weeks (Table 5.1; Figure 5.3). Temperature of incubation did not influence the average number of days to first ephyra release (P>0.05).

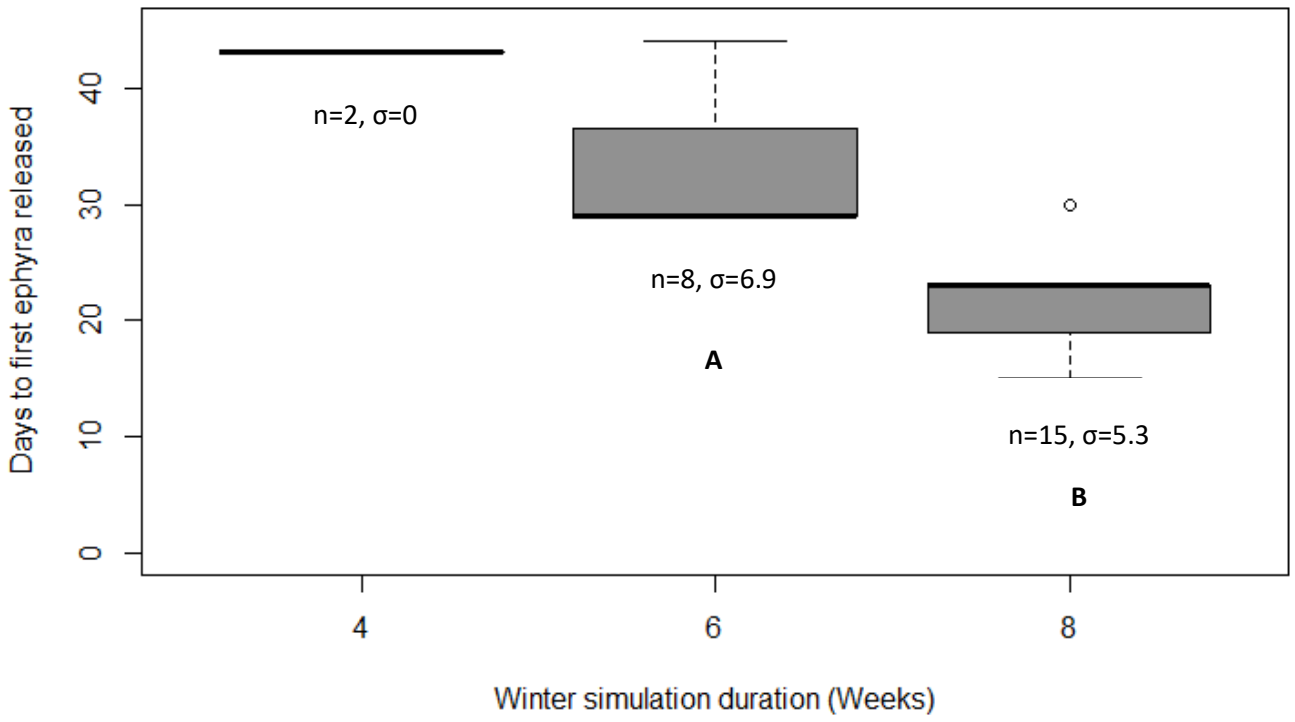

Figure 5.3. Number of days from the end of the winter simulation to first ephyra release. Only replicates that produced at least one ephyra are included. A bold line inside each box and whisker plot marks the median. The extremities of the box denote the first and

the third quartile and the dotted lines denote the range. Any points beyond the dotted line are outliers. Letters below each box indicates differences (e.g. A, B) between treatments, as determined by post hoc tests. Note the 4-week treatment was excluded from the analysis.

Table 5.1. Two-way ANOVA model results comparing the number of days to first ephyra release between treatments

Days to first ephyra release	**Mean Sq**	**df**	**F**	***P* value**
Duration	0.759	1	15.404	**< 0.001**
Temperature	0.066	1	1.348	0.259

Values in bold are significant at P<0.05. df = degrees of freedom

5.4.3 Number of replicates strobilating

More replicates strobilated in treatments incubated at 4 or 7 °C winter temperatures (Table 5.2). Approximately one quarter of replicates incubated at 4 and 7 °C strobilated, whilst only 4% strobilated at 10 °C (Figure 5.4a). Duration also affected the number of replicates that strobilated (Table 5.2), with more than 5 times as many polyps strobilating when incubated at winter temperatures for eight weeks than for 4 weeks (Figure 5.4b).

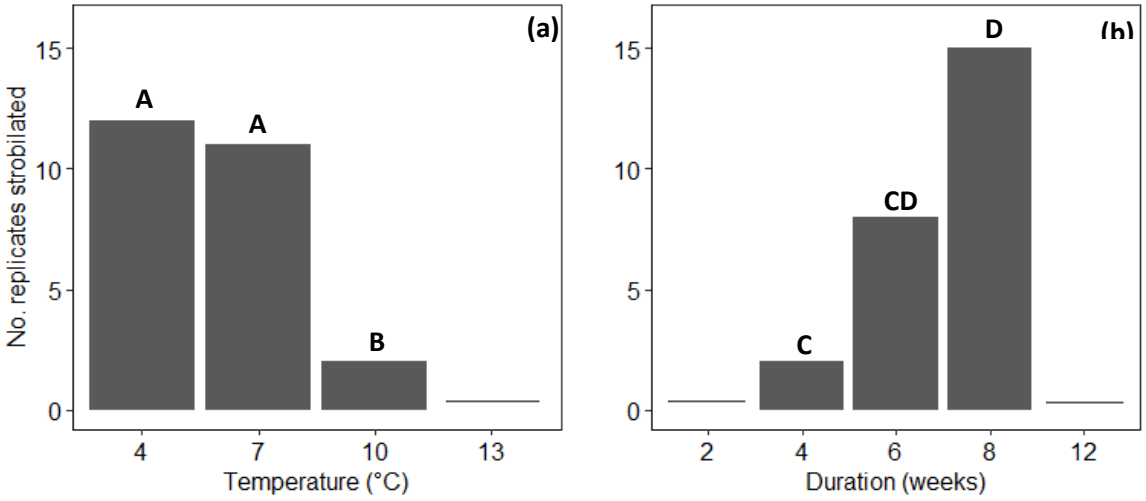

Figure 5.4. Number of replicates that strobilated at each (a) temperature and (b) duration. The two graphs refer to separate groupings and contain 120 experimental replicates and 40 control replicates, giving a total of 160 replicates each. Letters above data points

indicate similarities (e.g. A, A) and differences (e.g. A, B) between treatments, as determined by post hoc tests.

Table 5.2. Logistic regression model (family = binomial) test results comparing the influence of duration and temperature on the number of replicates strobilating within each treatment

% replicates strobilating within each treatment	Estimate	SE	*P* value
Duration	0.778	0.178	**< 0.001**
Temperature	-0.378	0.125	**0.002**

Values in bold are significant at P<0.05. df = degrees of freedom

5.4.4 Ephyra production

The number of ephyrae produced varied among duration treatments, but patterns differed depending on the temperature at which polyps were incubated (Table 5.3). For example, in the 6-week treatments more ephyrae were produced when incubated at cold or average temperatures, whereas those incubated at 10 °C only produced ephyrae when incubated for 8 weeks (Figure 5.5). The most ephyrae were produced in the 8-week treatment when incubated at 7 °C (with a maximum of 21 ephyrae produced by a single polyp).

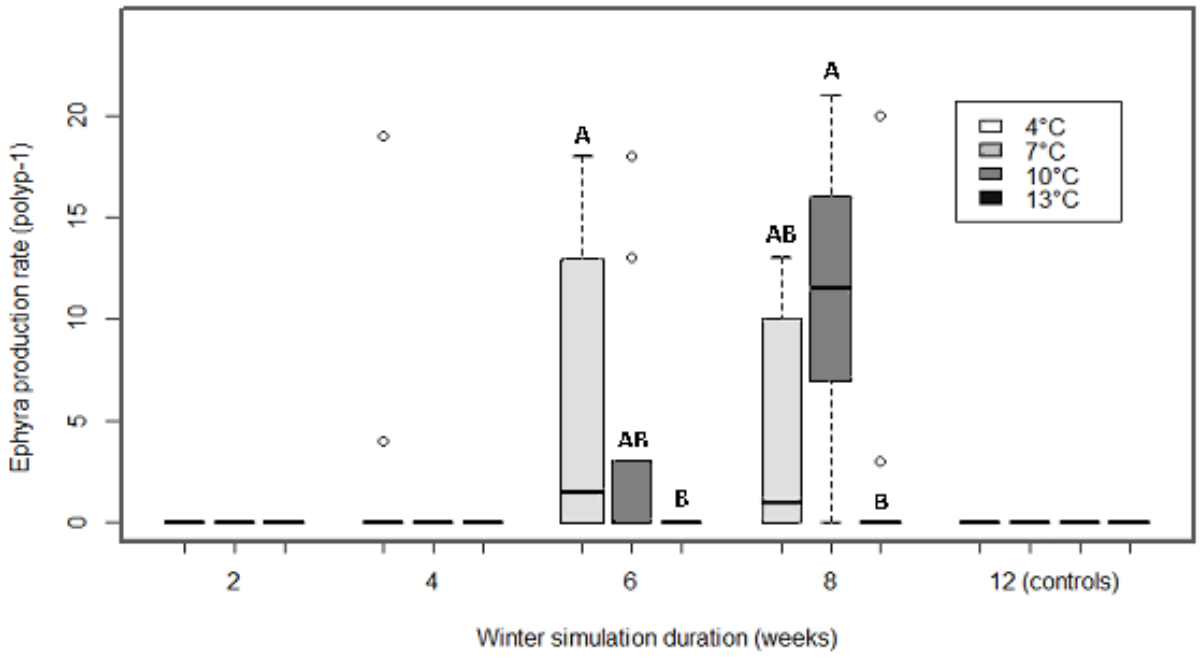

Figure 5.5. Median number of ephyrae produced per polyp in each treatment (n=10 replicates/treatment). A bold line inside each box and whisker plot marks the

median. The extremities of the box denote the first and the third quartile and the dotted lines denote the range. Points beyond the dotted line are outliers. Letters indicate similarities (e.g. A, A) and differences (e.g. A, B) between temperature treatments within each duration, as determined by post hoc tests

Table 5.3. Negative binomial regression model results comparing the total number of ephyrae produced per polyp between treatments (n=10). Values in bold are significant at p <0.05. df = degrees of freedom

Total ephyrae produced per polyp	**Estimate**	**SE**	**p value**
Duration	-1.291	0.914	0.158
Temperature	-2.323	0.934	**0.013**
Duration:Temperature	0.277	0.129	**0.032**

Values in bold are significant at P<0.05. df = degrees of freedom

5.5 Discussion

How changes in sea surface temperature may influence marine organisms, especially ectotherms (Pinksy et al., 2019) such as fishes (Moyano et al., 2017; Dahlke et al., 2020) and zooplankton (Edwards and Richardson, 2004; Kvile et al., 2016), has been one of the key focuses of research efforts over the past two decades. Whilst numerous studies have now examined temperature effects on jellyfish and especially on polyps (Shi et al., 2018; Purcell et al., 2012; Lu et al., 2020), none have examined potential interactions between temperature and duration of temperature on strobilation. Within this context, we show for the first time how the temperature and duration of the winter period influences reproduction in the spring. Polyps were more productive (i.e. more polyps strobilated and more ephyrae were produced) when incubated for longer periods of time at cold (4 °C) and average (7 °C) winter temperatures. Furthermore, polyps began strobilating sooner following longer (8 week) incubations. Consequently, the shorter, warmer winters predicted under climate change (IPCC, 2018; IPCC, 2019) are likely to produce fewer ephyrae and, potentially, to lead to smaller populations of medusae in temperate coastal regions. In the shortest incubation (2 weeks), no polyps strobilated and very few of those incubated at the warmest temperature (10 °C) strobilated and only when incubated for 8 weeks. This indicates that polyps are likely to have a limited thermal window in which they can successfully strobilate.

Thermal windows constrain ectothermic animal distributions and limit the geographic range within which they can live and reproduce (Höhn et al., 2017, Dahlke et al., 2020). Each population's thermal window differs, and this may contribute to the population-specific timings of reproduction, and to their variable responses to different minimum winter temperatures (Kroiher et al., 2000, Pascual et al., 2015, Kvile et al., 2016). Some studies report that warmer temperatures result in more ephyrae (e.g. Holst, 2012) and others report that cooler temperatures increase productivity (e.g. Kroiher et al., 2000, Purcell et al., 2012, Widmer et al., 2016). Such inconsistencies may exist because the *Aurelia* genus is highly plastic and the species *Aurelia aurita* comprises numerous cryptic species (Dawson et al., 2015). Our results from Horsea Lake on the south coast of the UK provide evidence that cooler winter temperatures could enhance strobilation in temperate populations of *A. aurita* polyps.

Interannual variations in timing of strobilation result partly from differences in winter duration and temperature. In the laboratory, the 'preconditioning period', experienced by polyps prior to the induction of strobilation, mimics this preparatory period and influences how polyps respond to cues. Differences in preconditioning between experiments may explain variations in response time and production of ephyrae between these studies (Kroiher et al., 2000, Purcell et al., 2012, Fuchs et al., 2014). However, due to large differences in experimental setups and procedures, separating this influence from other environmental factors in past studies is very challenging (Hubot et al., 2017). In situ, the trend of delayed ephyrae appearance after cold winters observed in Southampton Water by Lucas (2001) can be partly explained by the duration of the winter period and, in particular, the timing of the winter minimum. Shorter, sharper winters, whilst increasing the final number of ephyrae produced, may delay their production as compared to longer winters where ephyrae respond more rapidly to the eventual warming of the water in the spring.

A key finding of our study was that minimal strobilation was observed following incubation at warmer-than-average winter temperatures. The interaction of the RxR transcription factor and the spp. specific protein CL390, a likely candidate for the strobilation hormone, provides insight into the molecular processes behind the lack of strobilation in the warmer treatments (Fuchs et al., 2014). Specifically, the above-average winter temperature may not have reached the critical low threshold needed to induce upregulation of the CL390 transcript. Alternatively, a longer incubation at these temperatures was needed to produce enough transcript to reach the activation threshold and initiate strobilation. This lack of strobilation has been observed in other experiments on temperate species, where temperatures were suggested to be too high to induce ephyra production (Willcox et al., 2007). This effect is compounded when examining temperature and duration combined.

Differences in the response to spring warming between the shorter (2, 4 weeks), and the longer winter incubations (6, 8 weeks) in the current experiment may represent the difference between the response to a short-term cold snap, and a seasonal change. In North Sea regions, such as along the French Normandy coast, polyps needed at least 15 days at colder temperatures when moved from 20 to 15 °C, and 9 days when moved from 18 to 10 °C to initiate strobilation (Kroiher et al., 2000). In Horsea Lake polyps, a longer winter incubation resulted in strobilation occurring more rapidly and, in more polyps, producing more ephyrae following the shift to spring temperatures. To initiate significant strobilation, polyps need to be incubated at an average or cooler than-average temperature for this region for about 6 weeks.

Most studies examining temperature effects on scyphozoan jellyfish have proposed that moderately warmer temperatures benefit temperate jellyfish populations, by enhancing ephyral growth and reproduction in polyps and medusae (e.g. Purcell, 2005, Widmer, 2005). These studies have focussed on the warmer (summer) temperature threshold. Warmer winter temperatures in temperate regions such as the North Sea, however, are likely to inhibit spring jellyfish blooms, as proposed by Lynam et al. (2004). The current study indicates that periods during winter where water temperature is at or below 7 °C for 6 weeks are likely to result in larger temperate *Aurelia* populations, whereas years that experience a warmer and shorter-than-average winter may experience reduced or delayed strobilation. However, it is still unclear whether induction of strobilation occurs due to reaching a critical temperature threshold (e.g. winter minimum) or a relative change in temperature, and it would be beneficial to understand how consistent these results are between different populations. This study has important implications in the current climate of global warming, where some regions are experiencing rapid warming and increasing winter temperatures (Belkin, 2009). For example, enclosed and semi-enclosed European seas such as the Baltic and North Seas have experienced rapid warming from 1982 to 2006, with the Baltic Sea warming at a rate of 1 °C per decade (Belkin, 2009). Holst (2012) posited that increasing sea temperatures might benefit *A. aurita* polyps from the North Sea.

5.6 Conclusions

This study shows that polyps from Horsea Lake, UK, are unlikely to strobilate after experiencing warmer-than-average winter temperatures. In future scenarios of climate warming, temperate polyps are likely to experience shorter, warmer winter periods that may not always reach the long-term average winter minimum. Whilst there are many confounding factors that drive jellyfish population cycles and the appearance of blooms (Brodeur et al., 2008; Lynam et al., 2011), if polyps no longer experience the cues to initiate strobilation, then they are unlikely to produce ephyrae, preventing future bloom formation (Fuchs et al., 2014; Treible and Condon, 2019).

Despite evidence that some local populations may be able to adapt to changing environmental conditions (Lu et al., 2020), recent warming may be too fast for these species to respond to (IPCC, 2018). Future investigations should focus on this crucial preconditioning period by studying each life stage in isolation and in combination with others, to predict how changing conditions will affect jellyfish populations in the future.

5.7 Supplementary information on *Chrysaora hysoscella*

Six species of scyphozoans are indigenous to UK coastal waters such as the moon jellyfish *Aurelia aurita* and the compass jellyfish *Chrysaora hysoscella* (Russell, 1970), with most sightings of the medusae reported between May to September (Pikesley et al., 2014). From sightings reported as part of the Marine Conservation Societies' "Jellyfish" Survey from 2003-2011, the majority of *Ch. hysoscella* sightings were concentrated around the southeastern coast of the UK, and sightings become rarer further North (Pikesley et al., 2014). Despite its global presence, this species is still relatively unexplored as compared to *Aurelia* ssp. Studies on the medusae life stage are far more common than on the polyp (Mariottini and Pane, 2010; Piksley et al., 2014). The medusae is known to have a global distribution from the North Sea (van Walraven et al., 2020), to the Mediterranean Sea (Del Negro et al., 1990), as well as in northern Namibia to Cape Town (Sparks et al., 2000), where it is purported to live at temperatures from 4 to 28 °C. However, in UK coastal waters and in the North Sea, polyps of this species have never been located *in situ* (van Walraven et al., 2020), with experiments examining the polyps using planula larvae or stock polyps (Widmer et al., 2016).

The aim of the following experiment was to explore whether polyps of *Ch. hysoscella* could survive and reproduce in realistic temperatures chosen to reflect winter temperatures in UK coastal waters. The following hypotheses were tested: differences in the duration and temperature of the winter period would significantly affect (i) the time from the temperature increase to strobilation, (ii) the proportion of polyps strobilating, and (Bayha et al., 2012) the total numbers of ephyrae produced.

Polyps of *Ch. hysoscella* were taken from stock held at the National Oceanography Centre Southampton. Feeding and maintenance were the same as for the previous experiment (see section 5.3.2). Experimental design was also identical to the experiment detailed in the previous sections on *Aurelia aurita* (Figure 5.1).

Survival rates were low in all treatments exposed to 4 °C, no matter the length of exposure, ranging from 60-80 % mortality across the treatments. Survival was highest at the warmest

temperature (10 °C), with between 0 and 20% mortality across all treatments. No budding or strobilation was observed in *Ch. hysoscella* polyps at any temperature across the experiment.

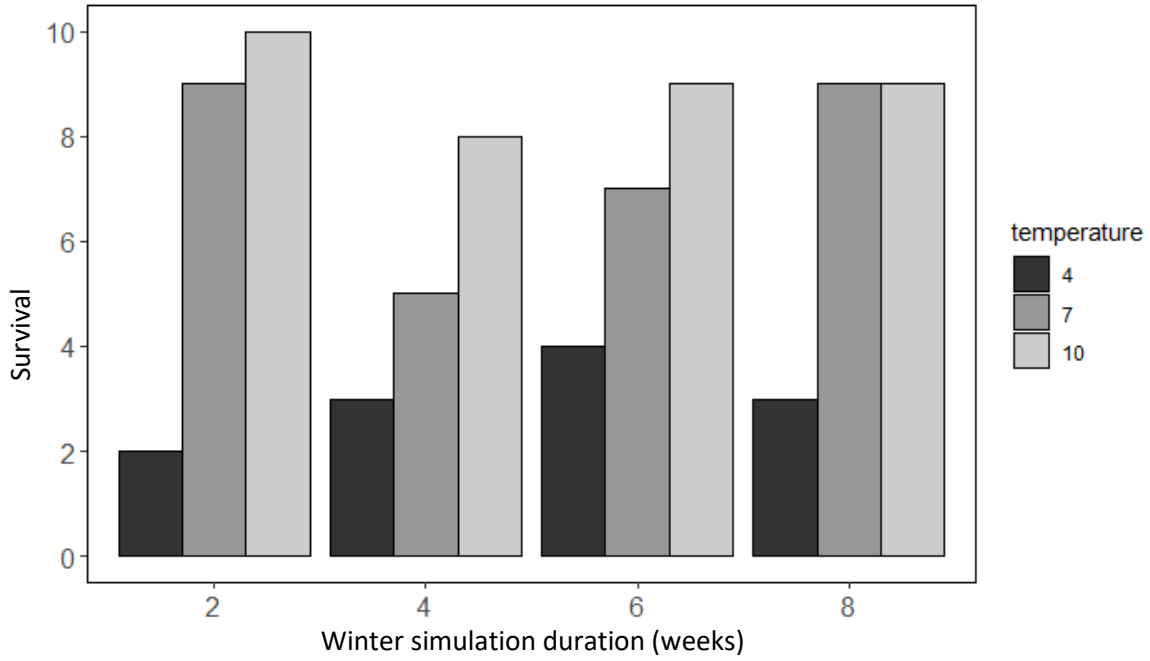

Figure 5.6. Number of *Ch. hysoscella* polyps alive at the end of each experiment

Chrysaora hysoscella medusae are a common feature of southern UK coastal areas in the summer months (Pikesley et al., 2014). However, our data shows that following varying durations at realistic winter temperatures, neither the duration nor the temperature of incubation affected strobilation or budding, as polyps did not produce any offspring across any of the experiments. In many species such as *Aurelia* sp., warmer thermal regimes increase polyp budding rates (Purcell et al., 1999; Han and Uye, 2010; Hubot et al., 2017), and our data supports this observation. As well as this, there was a large increase in mortality at the coolest temperatures, which supports the idea that 4 °C is too cold for *Ch. hysoscella* polyps, potentially representing a bottleneck in this species' lifecycle. High mortality at the lowest temperatures (Figure 5.6) agrees with previous experiments where *Ch. hysoscella* suffered 100% mortality at 4 °C (Widmer et al., 2016). In that experiment, highest rates of budding were reported at 23 °C, a much higher temperature than any tested in this experiment (10 °C maximum). If populations are present in the UK, budding may primarily occur in the warmer summer months, replenishing the benthic population from losses incurred during colder periods.

The surviving polyps did not respond to the increase in temperatures simulating spring warming and did not produce any ephyrae. Observation on timing of ephyrae release in natural population are lacking in *Ch. hysoscella* (Widmer et al., 2016). In past experiments, strobilation peaked at 14 ° C, which is very close to our target temperature of 13 °C, however we observed no

strobilation across any of our treatments. As well as this, we hypothesise that our simulation temperatures were too cold, as ephyra production is enhanced when polyps are maintained at warmer winter temperatures such as 10 °C (Holst, 2012), conversely to *A. aurita*, which benefits from a period at colder temperatures.

In scyphozoans, the medusa represents the primary dispersal mode, and can be dispersed vast distances following currents and other hydrographical features. Another explanation for the high mortality rate and lack of strobilation could be that that medusae seen in UK coastal waters may have been dispersed from areas with warmer temperatures, as polyps may not be able to survive colder UK winters. In accordance with (Widmer et al., 2016), the range of *Ch. hysoscella* may increase under future warming scenarios. However, this species is comparatively understudied so more information would be necessary before drawing any robust conclusions from this work. This experiment has highlighted the need for research to not only focus on a single model species such as *Aurelia* spp., but to also examine other indigenous species, which despite similar timing in appearance, may respond very differently to environmental change.

Chapter 6 General discussion

6.1 Background

Interest in understanding the drivers behind jellyfish life cycles has increased over recent decades, partly due to highly visible and sometimes disruptive blooms, most of which occur in coastal areas (Amorim et al., 2018; Kingsford et al., 2018). These spectacular aggregations of medusae occur at global, regional and local scales (Flynn et al., 2012; Richardson et al., 2009), frequently disturbing human activity with consequences ranging from stings and closed beaches (Kingsford et al., 2018), to clogged fishing nets (Roux et al., 2013), damaged aquaculture stock (Dong et al., 2017) and blocked power station cooling water intake valves (Rajogopal, 1989). However, increased examination of marine ecosystems in recent years has led to a paradigm shift in our understanding of the services that jellyfish provide (Doyle et al., 2014; Yusuf et al., 2018). They are no longer regarded as trophic dead ends, rather as key players in many ecosystems; for example acting as food items for over 100 species of fish and obligate gelatinous predators, providing shelter for juvenile fish as well as shunting carbon from the upper ocean to the deep ocean (Yamamoto et al., 2008; Lebrato et al., 2012). As well as this, products harvested from jellyfish provide ingredients for pharmaceutical and industrial applications (Leone et al., 2015), and medusa locomotion continues to inspire biomechanical design (Nawroth et al., 2012). Despite fulfilling various ecological roles, jellyfish remain comparatively understudied compared to other marine classes and there is still no full understanding of the main drivers behind the timing and size of jellyfish bloom appearances.

There are multiple reasons that explain this lack of knowledge. Jellyfish are not a homogenous group as once believed, but a complex group of animals whose responses to environmental stressors differ across species and life stages, as well as between geographical regions (Dawson, 2005a; Dawson and Martin, 2001). Most bloom-forming scyphozoan jellyfish have complex life histories involving an asexual benthic polyp and a sexual pelagic medusae (Lucas, 2001). The cryptic appearance of the polyp makes species identification in the field extremely difficult (Calder, 1971; Straehler-Pohl and Jarms, 2010). Each of the four scyphozoan life stages (medusa, planula larva, polyp, ephyra) fulfil different functions in the life cycle, and the two “adult” stages, the medusa and polyp, live in very different environments (Lucas, 2001). As such, elucidating what drives the population dynamics, both within and between life stages, will help researchers to understand how environmental variables drive jellyfish lifecycles and potential bloom formation.

This is especially important in the context of global climate change, which has been proposed to benefit gelatinous animals such as jellyfish (Holst, 2012). Analysis of the recent literature indicates that jellyfish blooms may be increasing in size and frequency due to anthropogenically-induced climate change (Condon et al., 2012; Lynam et al., 2011; Richardson et al., 2009; Richardson and Gibbons, 2008). However, as of yet these claims are unsupported by long-term observational and experimental data (Pitt et al., 2018; Greenberg, 2009). The changes associated with climate change may leave some populations vulnerable and could benefit one life stage disproportionately to another. It is therefore important to understand how human mediated climate change may affect scyphozoan jellyfish life stages and population cycles. Within this overall context, this thesis draws attention to the main questions identified in the introduction:

(i) How important is each life stage is to the overall population cycle and how vulnerable is each they to environmental stressors?
(ii) How do demographic differences in survival, growth and fecundity translate into visible jellyfish outbreaks?
(iii) What is the prognosis for populations under future climate change?

To answer these questions, we chose the common, or moon jellyfish *Aurelia aurita* as our primary experimental organism. The genus *Aurelia* is a wide-ranging cosmopolitan group composed of many sub- and sister-species (Schroth et al., 2002b; Dawson and Jacobs, 2001; Dawson and Martin, 2001). Its flexible life traits and ecological plasticity have enabled it to become endemic in ecosystems around the globe (Dawson and Martin, 2001). As such, it is the best-studied genus in the Scyphozoa, yet much remains unknown in terms of life stage transition and population cycle drivers. In the coastal waters surrounding the United Kingdom, *A. aurita* is one of the most common scyphozoans observed in summer months (Lucas et al., 1997; Russell, 1970). As well as this, *A. aurita* presents a 'typical' metagenic scyphozoan lifecycle composed of four life stages (Goldstein and Steiner, 2020). By studying this organism, we can broaden the applicability of our results. Nevertheless, as jellyfish are not a homogenous group, we also examined facets of other species to temper this approach, including the *Catostylus mosaicus* medusa and the *Chrysaora hysoscella* polyp life stages. By choosing to expand our research to other species, we demonstrate how other species may vary in both environment and response to forcing factors as compared to a 'typical' model species.

The following discussion summarises how each of the data chapters in my thesis have contributed to reducing these knowledge gaps and concludes by highlighting future research directions.

6.2 Functions of scyphozoan life stages and drivers of life-stage transition

6.2.1 Medusa life stage

In brief:

- Provisioning of planula larva by the medusa partly determines larval residence time in water column, but has no effect of settlement or growth
- Human infrastructure modifying freshwater flow into estuaries changes the population dynamics of native medusae

From a socio-economic perspective, the medusa is the most important life stage (Kingsford et al., 2018). The use of medusa-derived products in industrial and pharmaceutical applications (Brotz et al., 2017), as well as the disruptive nature of jellyfish blooms in coastal areas (Kingsford et al., 2018) means that understanding what drives medusa distribution, abundance and phenology is critical. Present typically across a few months to a year, the two main functions of the medusa life stage include (I) the production of planula larvae via sexual reproduction, and (II) long-distance dispersal, determining where larvae are released.

Medusae employ an r-strategy when producing gametes, typically producing multiple thousands of larvae at the expense of reduced parental investment in the offspring (Lucas 1998). Sexual reproduction enables genetic mixing within the population, simultaneously increasing population stability and resilience to environmental change (Lloyd, 1980). Production mode varies depending on the species of scyphozoan, from fertilising and brooding the eggs in the female, to releasing unfertilised eggs into the water column (Lucas and Lawes, 1998; Holst et al., 2007). Despite the different methods of production, without sufficient maternal provisioning larvae are likely to die before settlement or end up choosing a sub-optimal settlement location (Gambill et al., 2018). How much an organism invests in reproduction is flexible, varying with both the condition of the organism and the external environment (Lucas and Lawes, 1998). In the Scyphozoa, investment is mostly driven by mesozooplankton abundance, however other variables such as temperature affect the subsequent settlement of the planula (Lucas and Lawes, 1998; Goldstein and Steiner, 2020).

In Chapter 3, we examined three life stages from a year-round population of *A. aurita* medusa. By collecting medusae each month instead of using laboratory-grown or single-collection specimens, we gained unique insights into how seasonal change affects the medusa-to-polyp transition, and

how it translates into offspring success or failure. We investigated how factors such as temperature and maternal provisioning affect larval settlement and growth of polyps. Alongside changing environmental temperature and salinity, medusa condition, size and maternal provisioning were recorded using egg and planula larva sizes as proxies. Female size at maturity, indicated by the presence of planula larvae in brood sacs, varied throughout the year, with minimum size at maturity differing by over 160 mm between April and December. Despite this, female size had no effect on survival, settlement success or growth, and egg size did not vary across the year, indicating that the environment into which the organism is released may play a larger role in successful settlement than maternal provisioning, provided the larva has a minimum resource level.

In our study at Horsea Lake, production of larvae was continuous across the ten study months. Survival of larvae did not vary significantly across the study period and we hypothesise that medusae have access to sufficient resources to reproduce near constantly, despite Horsea Lake typically presenting a poor food supply (Lucas et al., 1997). Despite constant production, planula larvae were significantly larger in May and July as compared to the rest of the year and there was a significant positive correlation between mean planula larva length and the number of days taken by a larva to settle. This agrees with past research in that larger, better provisioned larvae can persist longer in the water column before settling (Dong and Sun, 2018). Maternal provisioning may play a much larger role in the field, where increased dispersal may require larvae to persist for longer in the water column (Marshall and Keogh, 2003; Vodopivec et al., 2017).

As well as continuing the life cycle via sexual reproduction, the medusa life stage is the main mode of long-distance dispersal (Hamner et al., 1994; Vodopivec et al., 2017). Out of the three pelagic stages (medusa, ephyra, planula), the medusa is the longest lived, present for a few months to just over a year. Some populations, however, aggregate in specific areas (Hamner et al., 1994; Pitt and Kingsford, 2000). In a subtropical population of the estuarine jellyfish *Catostylus mosaicus*, we aimed to uncover which environmental variables influenced the presence or absence of medusae in a modified estuary. Using a long-term dataset with 11 different biotic and abiotic variables, salinity was identified for the first time as the main driver of medusae presence or absence in the estuary. Modification of freshwater flow by a barrage regulated the population dynamics of *Catostylus mosaicus.* Medusae were unable to survive extended periods at extreme low salinities such that they would experience in the estuary following a barrage opening. This research highlights the population-specific dynamics of scyphozoan medusae. Often, population cycles and responses to environmental drivers are highly contextual and can vary dramatically between populations (e.g. (Amorim et al., 2018).

In conclusion, the medusa stage is important to the overall population cycle in its two main roles of producing planula larvae and dispersing the larvae to potential new locations. Sufficient provisioning of planulae is advantageous, however due to the r-strategy employed by many medusae, variations in provisioning are unlikely to present a bottleneck in the jellyfish lifecycle. We would like to highlight that environmental and climate drivers are highly contextual and are specific to each population. In estuarine and coastal environments, modifications to natural systems through the construction of infrastructure regulating freshwater flows are highly likely to influence native jellyfish population cycles. Balancing environmental needs with socio-economic requirements is crucial to minimising the impact of increasing coastal and estuarine infrastructure around the globe (Airoldi et al., 2021).

6.2.2 **Polyp life stage**

In brief:

- Within the population's thermal range, *A. aurita* polyp growth and budding benefited from increased temperatures
- Within the population's thermal range transgenerational effects of temperature provided no benefit in warming scenarios
- A 'preconditioning' period at cooler temperatures prior to strobilation is necessary for polyps to produce offspring
- *Ch. hysoscella* polyps experience reduced survival and do not reproduce at typical UK winter temperatures. Polyp colonies may overwinter as podocysts.

In the last couple of decades, the polyp life stage has increasingly been recognised as a key determinant in the formation and maintenance of scyphozoan jellyfish populations (Lucas et al., 2012). The polyp has two main functions: (I) to maintain the benthic population via asexual budding and ensuring their survival through challenging conditions by producing podocysts; (II) to produce ephyrae via strobilation, both directly and indirectly influencing where medusae populations occur, the timing of their seasonal appearance and inter-annual variability in medusae abundance. For this reason, they are therefore critical to the success of a jellyfish bloom.

Polyps play an important role in replenishing the benthic population across the year through asexual reproduction (Treible and Condon, 2019; Schnedler-Meyer et al., 2018b). This confers a number of benefits to the population, including increased reproductive flexibility, enabling polyps to take rapid advantage of favourable conditions (Goldstein and Steiner, 2020). Budding is linked

directly to the *in situ* temperature experienced by polyps, and within thermal limits, budding rates increase at higher temperatures (Hubot et al., 2017). Chapter 4 confirms the clear effect of warmer summer temperature on polyp reproductive activity, increasing average output and reproductive rate. The temperate scyphozoan *A. aurita* is known to have a wide tolerance to different environmental conditions, and this is reflected in its flexible reproductive traits. It is able to survive overgrowth through production of stolonal buds (Feng et al., 2018), as well as producing a higher proportion of stolonal buds at higher temperatures (Hubot et al 2017). Our data also supports increased production of stolonal buds at higher temperatures, which could disperse the population over a wider area, creating space for offspring to produce direct buds. Additionally, we identified in Chapter 3 that early polyp growth rates are significantly positively correlated with temperature within polyps' thermal range, which is in line with other studies on polyp metabolic rates (Gambill and Peck, 2014).

Alongside direct thermal influences on polyp reproduction, we also investigated for the first time the maternal effects of temperature as a potential driver for acclimation to a warmer summer environment. Transgenerational effects did not offer any visible acclimation to warmer temperatures over the examined 4 °C range. Indeed, effects were most apparent in cooling scenarios: as polyps were cooled across subsequent generations, rates of reproduction were lower than those that remained at a cooler temperature across multiple generations. Seasonal cooling following the summer may therefore affect polyp colony growth in the autumn and winter period, due to a mismatch between parental and offspring thermal conditions. However, an increase in temperature across a wider temperature range (8 °C) has been shown to trigger early initiation of asexual reproduction and results in a larger reproductive output in the subsequent generation (Appendix 7.3.1, Lu et al. 2020). Whether or not this trend continues at temperatures beyond established thermal limits is unknown (Donelson et al., 2016).

Alongside its primary role of maintaining the benthic population, the polyp life stage produces ephyra following suitable environmental cues (Fuchs et al., 2014). Temperature has been investigated as a driver of strobilation (Treible and Condon, 2019; Shi et al., 2018), yet no studies have investigated how thermal 'preconditioning' of polyps influences reproductive output. In Chapter 5 we wanted to challenge the regularly-stated paradigm that warm conditions encourage jellyfish outbreaks by exploring how varying winter temperatures affect polyp strobilation following seasonal warming in the spring. Results of studies investigating how temperature affects strobilation have been inconsistent (Holst 2012; Treible and Condon 2019), with some studies proposing that polyps need a period of warmer temperatures before strobilating, and other proposing a period of colder temperatures is necessary. We determined that a period at cooler temperatures prior to strobilation was necessary for polyps to produce offspring. Polyps were

more productive when incubated for longer at colder winter temperatures (4 °C) and began to strobilate sooner following longer incubations (8 weeks). Indeed, polyps incubated for short durations (2 weeks), did not strobilate, and very few strobilated following incubation at the warmest winter temperature warmer temperatures (10 °C). Interannual variations in timing of strobilation, and therefore medusa blooms, results partly from differences in winter duration and temperature. Shorter, sharper winters, whilst increasing the final number of ephyrae produced, may delay their production as compared to longer winters where ephyrae respond more rapidly to the eventual warming of the water in the spring. This research contributes directly to the body of knowledge that aims to determine why interannual fluctuations in medusae abundance and timing occur, and reveals how temperature is a key driver behind polyp reproduction in temperate populations.

Due to their small size and cryptic nature, in many regions the location of many polyp colonies is unknown. Six species of scyphozoans inhabit UK coastal waters such as the moon jellyfish *A. aurita* and the compass jellyfish *Chrysaora hysoscella* (Russell, 1970). While *A. aurita* polyps have been observed *in situ*, the same is not true for *Ch. hysoscella* polyp colonies in UK coastal waters, and only three isolated polyps have been identified from samples collected on the Dogger Bank (van Walraven et al., 2020). *Ch. hysoscella* medusae are nearly always observed in southerly waters (Piksley et al., 2014), and this spatial range may reflect the availability of suitable thermal conditions (Holst, 2012). We aimed to determine whether it is likely for benthic populations of *Ch. hysoscella* to survive and thrive in UK coastal waters by exposing polyps to a range of realistic winter temperatures. As such, we can begin to determine polyp thermal limits and vulnerability to colder temperatures (Höhn et al., 2017). Low survival rates and a lack of any reproductive activity, including a lack of podocysts at any incubation temperature indicated that polyps were vulnerable to cooler temperatures. If populations exist in UK coastal waters, budding is highly likely to occur in the warmer summer months, replenishing the benthic population from losses incurred during colder periods. As well as this, production of durable, chitin-covered podocysts may enable the population to survive colder temperatures and periods of extreme environmental conditions (Arai, 2009). We hypothesise that the vulnerability of polyps to colder temperatures may represent a bottleneck in the *Ch. hysoscella* life cycle.

In conclusion, the polyp stage is important to the overall population cycle because this life stage directly influences where and when ephyra are produced, and as such, when and where blooms will occur. Polyps play an important role in replenishing the benthic population and ensuring the continuation of the life cycle through inhospitable conditions. We identified temperature as a key influence driving polyp reproductive activity, increasing reproductive rates and production of different modes of reproduction at higher temperatures. We determined that warmer winters

may prove to be disruptive to the production of ephyra and may result in delayed or reduced blooms of *A. aurita* medusae, however colder winters may not benefit all species present in UK coastal waters.

6.2.3 **Planula larva life stage**

In brief:

- Sufficient parental provisioning is key to larval survival
- Unlike later growth rates, early growth rates are driven by the *in situ* thermal regime

With a strong focus on investigating the medusa and the polyp life stages, the "juvenile" stages, those that transition between the medusa and polyp, are very understudied, despite their importance to the overall life cycle. The planula larva has two main functions: (I) to disperse sexually-produced gametes to a successful settlement location on the benthos, and (II) to link the pelagic and benthos by metamorphosing from a relatively simple larva into a viable polyp.

As a simple non-feeding stage, the planula relies on adequate maternal provisioning from the parent medusa (Lucas and Lawes, 1998; Schneider and Weisse, 1985). It remains highly vulnerable to environmental stressors during its short lifespan of a few hours to a few weeks (Schneider and Weisse, 1985). Survival rates are typically very low, even in laboratory experiments where factors such as predation and unfavourable currents are removed (Dong and Sun, 2018). In Chapter 3, we examined planula settlement across a year. In our individual settlement experiments fewer than 10% of planula survived from release to polyp maturity. As an r-strategist, high fecundity and little parental investment in larvae by the medusa results in the production of thousands of offspring that are likely to disperse to a variety of substrates, with no guarantee of a suitable settlement location (Lucas and Lawes, 1998; Lucas, 1996). We demonstrated that planula larvae size and therefore maternal investment was strongly positively correlated with the number of days from release to settlement. The planula is strongly reliant on the medusae for survival as the longer a larva can persist in the water column, the more time it has to find a suitable settlement location (Schneider and Weisse, 1985). There is a fine balance to be maintained however, as the planula risks exposure to both biotic and abiotic stressors such as being eaten or carried away from a suitable settlement location. Our results also indicated that more larvae survived when settling with conspecifics rather than when settled individually.

Early growth of the polyp, prior to the start of feeding, is determined both by the larval condition and the environment in which it settled. In our experiments not all planulae that settled grew to polyp maturity and we hypothesise that some larvae will have exhausted their resources by the

time of settlement (Schneider and Weisse, 1985). Without sufficient energy to produce the first few tentacles, it would be unable to replenish its energy by feeding and will not survive. However, this could also be a consequence of the medusae investing too few resources into the gamete, combined with the planula being too 'choosy' and taking too long to settle (Pechenik, 1999; Brewer, 1978; Marshall and Keogh, 2003). Alongside this, we identified temperature as a key determinant in early polyp growth prior to the start of feeding. Newly metamorphosed polyps exposed to warmer temperatures grew their first eight tentacles more quickly than those exposed to cooler temperatures. Later polyp growth rates, from eight tentacles to maturity, were not related to their thermal regime, and we hypothesise that once polyps start to catch prey and feed, growth becomes more complex with both food quantity and quality determining how quickly a polyp grows to full maturity.

In conclusion, the planula larva stage is important to the overall population cycle because it provides the link between the pelagic and the benthic life stages by dispersing sexually produced gametes to the benthos. Due to the high volume of larvae produced and it being a non-feeding stage, it is unlikely to represent a key bottleneck in continuation of the scyphozoan population. Nonetheless, as polyps are sedentary, the location chosen by the planula will indirectly influence where blooms occur. Temperature remains a key variable influencing settlement and early growth of polyps, however it is highly likely that other factors, such as predation and currents are equally, if not more important in determining the success of this life stage.

6.2.4 **Ephyra life stage**

In brief:

- Rapid production of ephyra by polyps following environmental change will allow ephyrae to take advantage of beneficial conditions

As with the planula larva, the main function of the ephyra is to link the benthic and pelagic stages, from polyp to medusa. Survival of the ephyra is largely determined by the polyp, which determines the timing of release. However once released into the water column, like the planula, the ephyra is highly vulnerable to environmental variability as it cannot swim very powerfully until it develops into a medusa (Wang and Li, 2015; Fu et al., 2014; Feitl et al., 2009). The length of this life stage also varies greatly depending on the environmental conditions surrounding its release (Fu et al., 2014). The ability of the polyp to take advantage of rapidly changing conditions can give ephyrae an advantage over other marine species that take longer to develop and release their offspring (Båmstedt et al., 2001). Compared to the planula, the ephyra life stage has been

examined to a greater degree, as it is a useful ecotoxicology model, and has provided insights into how development occurs in gelatinous organisms (Spangenberg et al., 1994; Dong et al., 2017; Faimali et al., 2014).

In conclusion, the ephyra stage is important to the overall population cycle because it is the link between the benthic and pelagic life stages, however, as with the planula, it is unlikely to represent a critical bottleneck in the scyphozoan life cycle, despite its vulnerability to environmental variation.

6.2.5 **Which life stage represents the critical bottleneck in the jellyfish lifecycle?**

Through four research projects we have demonstrated the intricate complexity of the jellyfish life cycle. We explored how each life stage responds differently to environmental stressors on various spatio-temporal scales and have highlighted the vulnerability of each life stage to important environmental stressors such as salinity and temperature. As well as this, we indicated potential key bottlenecks in the jellyfish life cycle. Finally, we have begun to link different life stages together by indicating how the success of one life stage impacts the next, as well as examining factors that may mitigate stressors such as maternal effects and adequate larval provisioning. To conclude this section, out of the four life stages, and within the context of the environmental variables examined in this work, we believe the transition between the polyp and the ephyra represents the most critical bottleneck in the jellyfish lifecycle. In the next section, we explore this idea by exploring how demographic differences in survival, growth and fecundity translate into visible jellyfish outbreaks.

6.3 How do demographic differences in survival, growth and fecundity translate into visible jellyfish outbreaks?

Our findings suggest that the thermal regime experienced by scyphozoan jellyfish, particularly the polyp life stage, is likely to act as a major constraint on the size and timing of jellyfish blooms. To our knowledge, this body of research presents the first comprehensive quantitative study of how changes to thermal regimes modify the population demographics of scyphozoan populations. As ectothermic organisms, jellyfish metabolic and reproductive processes are intrinsically linked to the seasonal thermal changes that they experience across the year (Goldstein et al., 2017; Holst, 2012). For example, warmer temperatures in the summer are linked to increased planula longevity times in the pelagic, and increased reproductive outputs within a population's thermal range.

The size and timing of jellyfish blooms are a natural consequence of a complex life cycle encompassing both the pelagic and benthic sphere (Sukhoputova and Kraus, 2017; Goldstein and Steiner, 2020). Simply put, the interaction between an organism and its environment determines the differences in survival, growth, and fecundity across the year. This body of work highlights the individual importance of each life stage ensuring the survival of the population in changing conditions, however, it also brings attention to the potential bottlenecks in the life cycle, such as the polyp-to-ephyra transition. Here, increased winter temperatures result in delayed and reduced production of ephyrae following spring warming. As well as this, thermal regimes, which benefit some species, may not prove beneficial to others, with *Ch. hysoscella* polyps suffering reduced strobilation and increased mortality at temperatures that would benefit *A. aurita* strobilation.

Asides from the environmental context in which an organism lives, the environment experienced by previous life stages tempers an organism's response to a varying environment. This ranges from differences in maternal provisioning by the medusa, determining larval longevity and survival; to the choice of settlement location by the larva, indirectly determining where medusa blooms will occur following polyp strobilation. The environmental regime experienced by a parent can also carry over to the offspring, however this is not always necessarily environmentally adaptive, with no acclimation to a warmer environment over the studied temperature range.

Finally, animals have evolved to fill specific ecological niches within their environment. However, human activity can disrupt animal activity and modify populations (Amorim et al., 2018; Vodopivec et al., 2017). For example, medusa survival in the Fitzroy estuary is driven by freshwater inputs from the barrage located 60km upstream. As well as this, building this infrastructure has modified the population range, limiting the medusae to below the barrage. As with many polyp populations, the location of the benthic stages is unknown, and further research is required to understand whether polyps able to persist in the conditions experienced by the medusae or if they are also vulnerable to freshwater inputs. Other human mediated changes to the environment, such as climate change, are discussed in the next section.

6.4 Prognosis for the future

Anthropogenically forced climate change is driving changes to the ocean system, from increasing global mean temperatures, sea level and the frequency of marine heatwaves, to increasing ocean acidification and changes to species distribution and phenology (IPCC, 2019; Belkin, 2009). Due to the complexity of both global climate change and the scyphozoan jellyfish life cycle, this thesis

focuses on increased temperatures as a main effect of climate change, due to the close links between temperature and ectothermic organisms such as jellyfish.

Much of the research examining temperature effects on scyphozoan jellyfish have proposed that moderately warmer summer temperatures benefit temperate jellyfish populations, by enhancing ephyral growth and reproduction in polyps and medusae (Purcell, 2005; Widmer et al., 2016). The results in all our temperature experiments contradict the trend in recent literature suggesting that *A. aurita* jellyfish benefit from anthropogenically induced climate warming. At temperatures within the population's thermal range, our results agree that warmer temperatures increase offspring production, growth and longevity. However, enclosed and semi-enclosed European seas such as the Baltic and North Seas have experienced rapid warming from 1982 to 2006, with the Baltic Sea warming at a rate of 1 °C per decade (Belkin, 2009). This trend is likely to continue under many predicted scenarios, resulting in temperatures that will increase past thermal limits.

Holst (2012) posited that increasing sea temperatures might benefit *A. aurita* polyps from the North Sea, due to potential future range expansion northwards. However, in future scenarios of climate warming, temperate polyps are likely to experience shorter, warmer winter periods that may not always reach the long-term average winter minimum. If polyps no longer experience the cues to initiate strobilation, then they are unlikely to produce many ephyrae, preventing future bloom formation (Fuchs et al., 2014; Treible and Condon, 2019). Despite evidence that some local populations may be able to adapt to changing environmental conditions (Lu et al., 2020), recent and predicted future warming may be too fast for these species to adapt (IPCC, 2019). As well as this, other confounding factors such as transgenerational effects that could potentially provide a buffer against rapid warming (Lu et al., 2020) do not appear to have any strong effects in warming scenarios within the 4 °C range studied.

It is important to note the variation in the response to temperature between species. For example, unlike *A. aurita*, *Ch. hysoscella* polyps may benefit from warmer winters in the future due to ocean warming, potentially resulting in range shift northwards. As such, the species composition of jellyfish found in UK coastal waters may change. However, only examining thermal conditions paints a limited picture of all predicted changes. As such, future research can build on the work presented in this thesis, to provide an increasing understanding of how predicted global warming will affect jellyfish.

Alongside a warming marine environment, increased instability in the ocean sphere is also predicted under future climate change (IPCC, 2019). In Chapter 3 we captured a snapshot of conditions across 2019, including anomalously high temperatures in July 2019. Our July temperature data may have been coincident with a short-term marine heatwave, although this

cannot be confirmed without temperature records across a period of five consecutive days or more (Hobday et al., 2016). Larvae settled from medusae collected in July spent more time in the water column than those collected in other months. Jellyfish species such as *A. coerulea* report negative effects of high temperatures on settlement, including smaller planula size and reduced survival rate (Dong et al., 2018). Planula larvae in July were significantly larger than other months and this may have also contributed to their longevity, however without data on the food conditions experienced by the medusae we cannot explain all the variation observed in the dataset (Goldstein and Steiner, 2020). Increased longevity of the planula in the water column is not necessarily beneficial to the larva, as the risks of predation and being swept away from a suitable settlement site increase with time (Brewer, 1984; Dolmer and Svane, 1993).

In conclusion, we predict that the thermal changes to the ocean sphere as predicted under future climate change will not benefit temperate scyphozoan jellyfish populations. Due to typically shallow depths and limited water exchange with the wider ocean environment (Lucas, 1996), coastal lagoons and sheltered inlets and fjords that often prove to be suitable environments for jellyfish and other animals such as juvenile fish, may be at increased risk from increased instability in the thermal regime. As ectothermic animals, increasing temperatures beyond their thermal limits will result in negative effects on health, survival and reproduction.

6.5 Research in practice

Asides from the scientific value provided in this thesis, it is important to highlight the wider applications of the knowledge to environmental management, policy and education. Human modification and management of natural systems is increasingly common in coastal areas (Lehner et al., 2011; Airoldi et al., 2021). In Chapter 2, we demonstrate how the construction of a barrage to mitigate flooding and store freshwater has dramatically changed the spatial and temporal patterns of occurrence of the estuarine medusa *C. mosaicus*. In highly managed estuaries, determining how species respond to modifications in their environment will enable stakeholders to balance environmental needs with socio-economic demands (Airoldi et al., 2021; Amorim et al., 2018; Bunn et al., 2010). For the first time, we demonstrated how modification of freshwater flow by a barrage modifies the population dynamics of native medusae. This research also highlighted the importance of collaboration between scientists and policymakers, with observational data supplied by the Queensland Department of Environment and Science. We suggested that smaller freshwater releases into the estuary would enable the medusae to survive with minimal impact, minimising the impact of human construction and activity on natural populations. By carrying out this research, we aim to support policymakers and managing organisations to work towards a sustainable management of global estuaries and waterways.

Alongside this, this body of research has also contributed to the developing discussion around whether jellyfish blooms are increasing due to human mediated climate change. Chapters of this research have been communicated to both specialists within the field, as well as to the general public, emphasising how jellyfish are not only important to human activity, but also to the correct functioning and increased biodiversity of many ecosystems. We have highlighted that they are not a single functional group, but instead are diverse in both form and function.

6.6 Recommendations for future research

Jellyfish population cycles and bloom events occur at global, regional and local scales, and understanding how the climate and the environment drives these cycles now and in the future is highly complex. In this context, this thesis has several strengths which enable us to provide a relevant and informative understanding of scyphozoan jellyfish life histories. Nonetheless, this approach is not without limitations. In this next section, the main strengths and limitations of this thesis are presented, with the goal of highlighting future avenues of research in a constructive manner.

Firstly, the balance of laboratory, aquarium and observational/field data utilised across the four data projects minimises the reliance on correlational and circumstantial evidence, enabling us to present robust, data-driven inferences (Pitt, 2018). Every project within this thesis is embedded in a realistic context, supplemented with observational environmental data that enables us to build a more comprehensive overview of each situation. Combining observational and experimental approaches such as in Chapter 2, firmly embeds experiments in an ecological context, yet allows researchers to explore responses in a controlled environment, and we would recommend this methodological approach where possible.

However, if anything, this research has highlighted how much is still unexplored when it comes to jellyfish life cycles. Within this thesis, there was a strong focus on temperature and salinity as the two main examined variables. These were chosen partly because of their relevance to climate change and ocean warming (IPCC, 2019), but also as they have been identified as two of the main variables driving population cycles in jellyfish (Fuchs et al., 2014), yet we still do not fully understand how they influence jellyfish life histories. We recognise that there are many important environmental drivers that still remain comparatively unexplored, such as food quantity and quantity, predation, and other trophic interactions.

We chose to approach this area at a population and individual level, as increasingly jellyfish are regarded as a heterogenous group made up of organisms filling various ecological niches, providing many ecosystem services in different environments (Graham et al., 2014). Even at

population level, much of the *in situ* research was carried out on the medusa life stage, primarily for logistical reasons. For example, we were unable to observe any polyp colonies *in situ*. By continuing to investigate the thermal limits of different populations in the laboratory, researchers may be able to narrow down where polyp colonies are located *in situ*, and as such, where jellyfish blooms may occur. As well as this, we recommend researchers investigate scyphozoan jellyfish at a molecular level, not only to identify cryptic lineages, but to also elucidate how gene expression varies in response to environmental stressors. This research is important to support population-level experiments, as they may present responses that only occur on a molecular level, and may represent the precursors to acclimation or visible stress indicators.

By maintaining a focus on the lower spatial levels, broader, global scales were not considered. Despite this, we believe that population and individual level experiments will be the most informative to researchers in this field in the current context. By investigating population-level responses in key locations, researchers can engage stakeholders and policymakers to inform sustainable environmental management, further highlighting the importance of jellyfish research. Such research has the potential to not only inspire and educate a new global generation of researchers, but to deepen our understanding of the ocean sphere and drive sustainable environmental management.

6.7 Conclusions

The research presented in this thesis advances our understanding of how the climate and environment affect jellyfish life stage transition, an aspect of scyphozoan jellyfish research that has been highlighted as are that requires further research. The research has:

- Identified key environmental drivers behind jellyfish life stage transition and population cycles;
- Highlighted the vulnerability of specific life stages to environmental variables;
- Demonstrated that responses to environmental factors are likely to differ depending on the species, temporal scale, and geographical location in question;
- And highlighted the vulnerability of temperate jellyfish populations to continued climate change.

Future research into jellyfish population cycles should continue to focus on life-stage transition, using a combination of observational and experimental studies to identify the key environmental drivers acting on different populations. Outputs from this research must move away from global-scale inferences on gelatinous zooplankton and instead must determine which populations are likely to be vulnerable to ongoing global climate change.

We are now only starting to understand the complex processes that drive scyphozoan population cycles. In order to minimise the impact of potentially harmful blooms on human activities, and inversely to reduce destructive human activity on delicate ecosystems, it is crucial that we continue to research scyphozoan jellyfish, as well as to engage stakeholders, policymakers and the public in the process of marine research.

Appendix A Supplementary information for Chapter 2

A.1 Environmental variables

Table A.1. Environmental variables collected by the Queensland Department of Environment and Science

Variable	Type	Unit	Sites measured at
Chlorophyll-a	Biological	µg/L	Upper, Mid, Lower
Pheopigments	Biological	µg/L	Mid, Lower
Total nitrogen (TN)	Physio-chemical	mg/L	Upper, Mid, Lower
Nitrogen ammonia (nh3)	Physio-chemical	mg/L	Mid, Lower
Oxygen	Physio-chemical	mg/L	Upper, Mid, Lower
Ph	Physio-chemical	Unit	Upper, Mid, Lower
Total phosphorous	Physio-chemical	mg/L	Upper, Mid, Lower
Salinity	Physio-chemical	n/a	Upper, Mid, Lower
Specific conductance at 25 °C	Physical	mS/cm at 25 °C	Mid, Lower
Turbidity	Physio-chemical	Nephelometric Turbidity Units (Bellantuono et al., 2012)	Upper, Mid, Lower
Secchi disk depth	Physical	meter	Upper, Mid, Lower
***Catostylus mosaicus* medusae**	Biological	Presence/absence	Upper, Mid, Lower

A.2 Correlation matrixes for (a) Upper, (b) Mid, (c) Lower estuary variables

Table A.2.a. Upper estuary correlation matrix

	Month	Year	Chlorophyll-a	Nitrogen Ammonia	Total Nitrogen	Oxygen	pH	Pheopigments	Total Phosphorous	Salinity	Temperature	Turbidity	Secchi Disk Depth	Specific Conductance at 25 °C	*Medusae presence /absence*	
Month	NA	-0.17	0.20	0.29	-0.49	0.57	0.63	0.24	-0.55	0.80	-0.42	-0.77	0.76	0.80	0.71	R values
Year	0.5424	NA	-0.29	-0.34	-0.31	-0.32	-0.23	-0.18	-0.32	-0.36	0.15	0.14	-0.24	-0.37	-0.10	
Chlorophyll-a	0.4849	0.2901	NA	-0.23	-0.26	0.72	0.55	0.81	-0.19	0.27	-0.07	-0.30	0.26	0.28	0.26	
Nitrogen Ammonia (NH3)	0.2866	0.2095	0.4063	NA	0.24	-0.12	-0.02	-0.37	0.00	0.52	-0.55	-0.37	0.42	0.52	0.38	
Total Nitrogen (TN)	0.0654	0.2542	0.3474	0.3854	NA	-0.45	-0.48	-0.33	0.96	-0.32	-0.04	0.45	-0.44	-0.32	-0.39	
Oxygen	0.0256	0.2480	0.0025	0.6821	0.0905	NA	0.93	0.72	-0.43	0.65	-0.23	-0.74	0.68	0.66	0.59	
pH	0.0110	0.4077	0.0351	0.9383	0.0687	0.0000	NA	0.52	-0.50	0.68	-0.35	-0.86	0.78	0.69	0.61	
Pheopigments	0.3879	0.5209	0.0002	0.1764	0.2279	0.0026	0.0492	NA	-0.20	0.22	0.10	-0.25	0.17	0.22	0.27	

	Month	Year	Chlorophyll-a	Nitrogen Ammonia	Total Nitrogen	Oxygen	pH	Pheopigments	Total Phosphorous	Salinity	Temperature	Turbidity	Secchi Disk Depth	Specific Conductance at 25°C	*Medusae presence /absence*	
Total Phosphorous	0.0332	0.2528	0.5010	0.9902	0.0000	0.1136	0.0551	0.4705	NA	-0.44	0.12	0.59	-0.58	-0.44	-0.50	R values
Salinity	0.0003	0.1826	0.3266	0.0483	0.2462	0.0082	0.0053	0.4345	0.0988	NA	-0.42	-0.88	0.91	1.00	0.87	
Temperature	0.1190	0.5988	0.7908	0.0334	0.9011	0.4153	0.2069	0.7360	0.6668	0.1211	NA	0.45	-0.52	-0.42	-0.33	
Turbidity	0.0009	0.6207	0.2737	0.1789	0.0904	0.0014	0.0000	0.3628	0.0210	0.00002	0.0924	NA	-0.95	-0.88	-0.80	
Secchi Disk Depth	0.0010	0.3860	0.3504	0.1237	0.0987	0.0050	0.0005	0.5383	0.0245	0.00000	0.0487	0.0000	NA	0.91	0.81	
Specific Conductance at 25°C	0.0003	0.1781	0.3166	0.0490	0.2473	0.0075	0.0047	0.4281	0.0990	0.00000	0.1152	0.0000	0.0000	NA	0.87	
Medusae presence /absence	0.0030	0.7185	0.3526	0.1593	0.1561	0.0206	0.0163	0.3215	0.0596	0.00002	0.2337	0.0004	0.0003	0.0000	NA	
P value																

Appendix A.2.b. Mid estuary correlation matrix

	Month	Year	Chlorophyll-a	Total Nitrogen	Oxygen	pH	Total Phosphorous	Salinity	Temperature	Turbidity	Medusae presence/ absence	
Month	NA	-0.21	-0.34	-0.51	0.69	0.67	-0.54	0.85	-0.59	-0.75	0.44	R values
Year	0.5375	NA	0.27	-0.33	-0.49	-0.40	-0.31	-0.38	0.23	0.22	0.04	
Chlorophyll-a	0.3085	0.4201	NA	-0.11	-0.32	-0.38	-0.08	-0.40	0.07	0.29	-0.26	
Total Nitrogen (TN)	0.1108	0.3207	0.7496	NA	-0.44	-0.45	1.00	-0.41	0.23	0.48	-0.23	
Oxygen	0.0180	0.1283	0.3348	0.1797	NA	0.95	-0.47	0.88	-0.59	-0.91	0.39	
pH	0.0227	0.2214	0.2485	0.1686	0.0000	NA	-0.49	0.88	-0.55	-0.93	0.43	
Total Phosphorous	0.0892	0.3560	0.8079	0.0000	0.1399	0.1289	NA	-0.45	0.25	0.53	-0.30	
Salinity	0.0010	0.2548	0.2200	0.2114	0.0004	0.0004	0.1604	NA	-0.62	-0.93	0.54	
Temperature	0.0561	0.4950	0.8342	0.5048	0.0585	0.0789	0.4579	0.0401	NA	0.54	-0.37	
Turbidity	0.0085	0.5098	0.3951	0.1349	0.0001	0.0000	0.0952	0.0000	0.0860	NA	-0.57	
Medusae presence/absence	0.1751	0.9168	0.4431	0.4928	0.2380	0.1818	0.3706	0.0862	0.2596	0.0647	NA	
						p values						

Appendix A.2.c. Lower estuary correlation matrix

	Month	Year	Chlorophyll-a	Total Nitrogen	Oxygen	pH	Total Phosphorous	Salinity	Temperature	Turbidity	Medusae presence/absenc	
Month	NA	-0.10	-0.36	-0.64	0.65	0.53	-0.67	0.77	-0.55	-0.63	0.22	R values
Year	0.7719	NA	0.41	-0.40	-0.24	-0.21	-0.30	-0.21	0.20	0.09	0.28	
Chlorophyll-a	0.2728	0.2081	NA	0.27	-0.66	-0.66	0.35	-0.62	0.17	0.68	-0.18	
Total Nitrogen (TN)	0.0323	0.2220	0.4211	NA	-0.71	-0.68	0.99	-0.74	0.24	0.69	-0.44	
Oxygen	0.0297	0.4768	0.0259	0.0138	NA	0.94	-0.80	0.96	-0.48	-0.94	0.28	
pH	0.0970	0.5407	0.0279	0.0207	0.0000	NA	-0.77	0.90	-0.29	-0.90	0.25	
Total Phosphorous	0.0232	0.3678	0.2921	0.0000	0.0029	0.0054	NA	-0.82	0.29	0.79	-0.45	
Salinity	0.0059	0.5447	0.0401	0.0090	0.0000	0.0001	0.0018	NA	-0.53	-0.93	0.32	
Temperature	0.0807	0.5511	0.6153	0.4788	0.1321	0.3861	0.3823	0.0939	NA	0.42	-0.32	
Turbidity	0.0373	0.7817	0.0225	0.0179	0.0000	0.0001	0.0039	0.0000	0.2013	NA	-0.42	
Medusae presence/absence	0.5184	0.4093	0.6023	0.1703	0.3977	0.4525	0.1630	0.3438	0.3348	0.1971	NA	

p values

Appendix B Supplementary information for Chapter 3

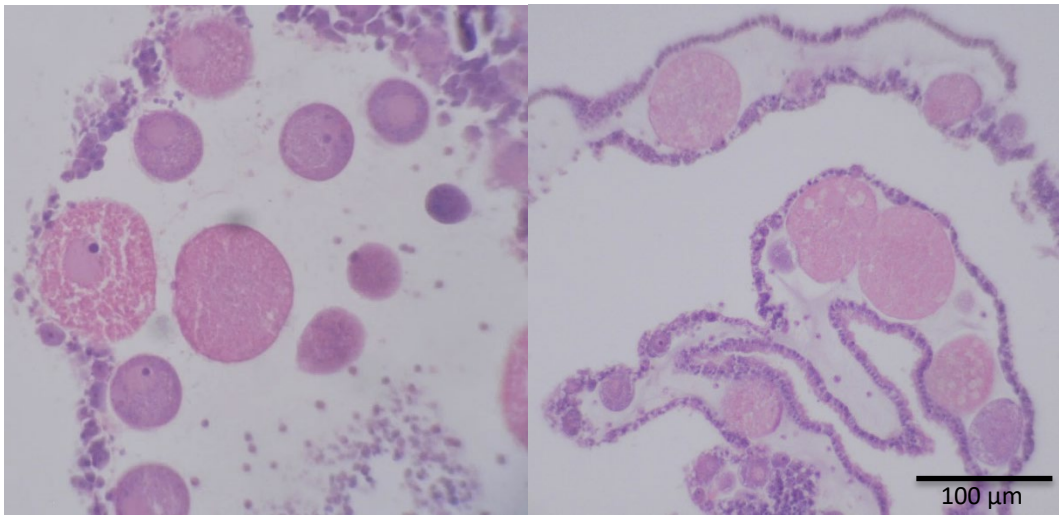

Figure 6.1. Examples of histological sections. Only oocytes with nuclei were measured

Table B.1. Results of the post hoc Tukey test determining significant differences between settlement rate of individually settled larvae across each monthly settlement experiment

	February	March	April	May	July	August	September	October
February	-	0.1638	1.0000	0.9999	0.0073	0.0001	0.9467	0.7532
March	-	-	0.1154	0.3748	0.9650	0.0000	0.0054	0.0009
April	-	-	-	0.9992	0.0043	0.0002	0.9749	0.8346
May	-	-	-	-	0.0297	0.0000	0.7712	0.4723
July	-	-	-	-	-	0.0000	0.0001	0.0000
August	-	-	-	-	-	-	0.0120	0.0498
September	-	-	-	-	-	-	-	0.9998
October	-	-	-	-	-	-	-	-

Appendix C Supplementary information for Chapter 4

C.1 Transgenerational acclimation influences asexual reproduction in *Aurelia aurita* jellyfish polyps in response to temperature

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Key Words: Transgenerational acclimation, Phenotypic plasticity, Jellyfish bloom, Invasive species, Temperature, *Aurelia aurita*

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This chapter is included in the supplementary material to provide further context to the research carried out in Chapter 4.

C.1.1 Abstract

Climate change events and anthropogenic activities (e.g. translocation of nonindigenous species) have been proposed to account for the rise of jellyfish blooms in coastal environments. Bloom-forming scyphozoan jellyfish of the genus *Aurelia* have successfully invaded new habitats and have caused damaging blooms. In attempting to understand the underlying reasons for their success, researchers have investigated immediate effects of changing environmental conditions (e.g. temperature) on scyphistomae of single/unknown generations, with a particular focus on asexual reproduction. However, it remains unclear how scyphistomae respond to changing conditions over longer time-scales or across generations, and how those responses influence bloom occurrence. Here, we examined the role of transgenerational acclimation in asexual reproduction of *A. aurita* scyphistomae in a 72 d orthogonal experiment, combining 3 parental with 3 offspring temperatures of 8, 12 and 16 °C. The null hypothesis was that the thermal history of the parental (F_0) generation will not affect asexual reproduction in the offspring (F_1) generation.

Our results indicated that, provided with a transgenerational temperature change, parent scyphistomae do modify the reproductive output and timing of offspring. Scyphistomae from 'cold' (8 °C) parents displayed the greatest reproductive output (2.86 buds per scyphistoma) and earliest budding commencement (23.86 d) at warm temperature (16 °C). Scyphistomae from 'warm' (16 °C) parents displayed the greatest reproductive potential (2.63 buds) at medium temperature (12 °C). Cold temperature (8 °C) caused considerable inhibition of asexual reproduction in offspring scyphistomae, independent of the parental thermal history. Transgenerational acclimation may benefit potentially invasive jellyfish species facing climate-related and/or human-induced changes in the global marine environment, by facilitating asexual reproduction and subsequent bloom events.

C.1.2 Introduction

As a critical component of marine biota (Hays et al., 2018), jellyfish populations that fluctuate periodically and often predictably are a natural feature of a healthy pelagic ecosystem (Graham et al., 2001). However, more spatially severe and temporally frequent outbreaks or blooms have been reported in some, but not all, regions of the world (Dong et al., 2010; Uye, 2011; Condon et al., 2012), several of which have resulted in potentially deleterious interactions with fisheries, tourism, and other human industries (Graham et al., 2014; Bosch-Belmar et al., 2017). While climate change events (Attrill et al., 2007) and human-induced causes, such as over-fishing (Lynam et al., 2011), eutrophication (Arai, 2001), translocation (Bayha and Graham, 2014) and marine urbanisation (Makabe et al., 2014; Vodopivec et al., 2017), have been proposed to account for recurrent jellyfish blooms (Kogovšek et al., 2018), robust quantitative evidence is often lacking (Pitt et al., 2018). In addition, the abundance of jellyfish populations often exhibits significant seasonal, inter-annual and decadal differences (Condon et al., 2013; Decker et al., 2014; Hosia et al., 2014), depending on scales of observations, making the possible causes even more complicated.

In the marine environment, life-history and behavioural traits that enable large numbers of individuals to be produced quickly, assuming high survivorship through to the adult phase, will predispose a species to form numerically large populations and potentially blooms or outbreaks (Lucas and Dawson, 2014). The bloom-forming genus *Aurelia*, like other members of the Scyphozoa, has a flexible life history, characterized by an alternation between sexual and asexual generations (Arai, 1997). The likelihood of anthropogenic introductions of *Aurelia* may reflect an elastic, flexible life history and ecology as well (Dawson and Martin, 2001), allowing it to be potentially invasive (Bayha and Graham, 2014). *A. aurita* is native to the NE Atlantic (Schroth et al., 2002a), but the genus *Aurelia* is also found in Korean waters (Park, 2000), the Inland Sea of

Japan (Uye and Shimauchi, 2005), the northern Adriatic Sea (Malej et al., 2006; Hubot et al., 2017), Chile (Häussermann et al., 2009), the Caspian Sea (Korsun et al., 2012) and many other coastal areas around the world (note that SE Asian and many other populations of *A. aurita* s.l. are now designated as *A. coerulea*). Molecular studies coupled with phylogenetic analyses have indicated the presence of as many as 13 locally adapted species of *Aurelia* worldwide (Dawson, 2003; Chang et al., 2016), of which 4 species appear to have been dispersed anthropogenically into distant water bodies (Bayha and Graham, 2014). This makes *Aurelia* an excellent model for studying causes of blooms created by exotic species.

Apart from the capacity for producing direct and/or stolon buds to increase their abundance, scyphozoan polyps can switch flexibly between up to 7 other reproduction modes, e.g. producing planuloid buds, motile bud-like particles, podocysts and free-swimming propagules (Adler and Jarms, 2009; Schiariti et al., 2014; Hubot et al., 2017). Relative longevity coupled with the potential to multiply in large numbers, and their capacity to withstand a wide range of adverse environmental conditions, make polyps a vital part of the scyphozoan life cycle. Of all the abiotic cues (e.g. oxygen, food supply, light, salinity) that influence polyp reproduction (Miller and Graham, 2012; Dong et al., 2015b; Chi et al., 2019; Treible and Condon, 2019), temperature appears to exert the greatest impact on polyps in terms of numerical growth rates (Ma and Purcell, 2005), survival (Chi et al., 2019) and the timing of strobilation (Fuchs et al., 2014). Furthermore, taking *Aurelia* sp. as an example, the actual temperature required for peak budding rates of a population shows species- and/or location-specific variability (Schroth et al., 2002a; Pascual et al., 2015; Hubot et al., 2017), indicating the occurrence of local adaptation (Lucas et al., 2012). While most studies have examined polyps in response to environmental cues in a single or unknown generation, little is known about the potential influence of conditions experienced by one generation on the growth and reproduction of the next generation.

The conditions experienced by parents can shape the performance of offspring via non-genetic maternal effects (or ‘parental effects’ for effects of a more general class) (Bernado, 1996; Marshall and Keogh, 2008) and/or epigenetic processes (Donelson et al., 2018). This ability, referred to as transgenerational acclimation or plasticity, can act as a key strategy to optimize individual fitness in fluctuating environments (Massamba-N'Siala et al., 2014) and an important mechanism for coping with rapid environmental variations across generations (Salinas and Munch, 2012; Donelson et al., 2018). In animal and plant ecology, the terms ‘transgenerational plasticity’ (TGP) as well as ‘parental’ and/or ‘maternal effects’ are usually reserved for sexual reproduction (Latzel and Klimešová, 2010). However, mounting evidence indicates that asexually generated offspring can also acquire adaptive phenotypic traits from their ancestor or previous generations (Latzel and Klimešová, 2010; Hafer et al., 2011; Norouzitallab et al., 2014; Garbutt et

al., 2014; Ye et al., 2019). Here, we use the term 'generation' as defined by the physiological individual (Latzel and Klimešová, 2010); in the case of budding propagation for scyphozoan polyps, an individual bud is considered as the offspring of the individual parent from which it is derived.

Introduced jellyfish species may possess a particular suite of traits that enhance 'invasiveness' and predispose them to exert significant ecological and economic impacts, e.g. in the form of a bloom (Bayha and Graham, 2014). Despite the large number of papers on jellyfish blooms in the last decade (see meta-analyses by Condon et al. 2012, Sanz-Martin et al. 2016, Pitt et al. 2018), studies examining the causes of blooms and research on transgenerational acclimation remain poorly integrated (but see Klein et al. 2017 on Irukandji jellyfish polyps). Consequently, we have limited knowledge on how the extrinsic characteristics of current ocean conditions and intrinsic traits of these influential species interact to cause jellyfish blooms in exotic habitats. In the context of rapid climate change and extreme climate regimes occurring on a global scale (IPCC, 2018), it is essential to investigate how marine species may respond to predicted environmental conditions via transgenerational studies under a 'winners and losers' scenario (Byrne and Przeslawski, 2013; Torda et al., 2017).

Here, we aimed to investigate whether TGP exists in the widespread bloom-forming jellyfish *A. aurita*, and to determine the role this may have in potentially acclimating polyps for predicted (elevated/reduced) thermal conditions. For this purpose, asexual reproductive traits of 2 generations (F_0, the mother generation; and F_1, the offspring generation) of polyps were tested at 3 temperatures (8, 12, 16 °C). Horsea Lake (UK), the source of the medusae used to generate F_0 scyphistomae, has a seasonal temperature range of 5.5−22 °C (Lucas, 1996; Hoehn, 2017). According to a 12-month SCUBA photographic survey in Horsea Lake, asexual budding occurs during the spring to autumn months, peaking at temperatures around 16 °C (D. Hoehn unpubl. data). Hence, our experiment was confined to temperatures experienced in the spring and autumn period (i.e. 8−16 °C), omitting both the lowest winter and highest summer temperatures when strobilation or podocyst production may dominate. The null hypotheses were that environmental temperature does not affect (1) the asexual reproduction strategy of *A. aurita* polyps (i.e. reproductive mode and proportion of buds produced by each mode), (2) the total number of buds produced per polyp (i.e. reproduction rates) and (3) the timing (i.e. initiation) of asexual reproduction, when parents were exposed to different temperatures.

C.1.3 Methods

Animal origin and acclimation

Scyphistomae of *Aurelia aurita* used in this experiment originated from reproductively ripe female medusae collected in January 2018 from Horsea Lake, an enclosed, brackish body of water on the south coast of England (50.8368° N, 1.1019°W), where temperatures range from 5.5 °C in February to 22.0 °C in August (Lucas, 1996; Hoehn, 2017). Planula larvae released from the oral arms of several individuals settled on petri dishes and metamorphosed into scyphistomae after 5−7 d. The newly settled scyphistomae were maintained in a plankton kreisel tank for 1 month at a mid-range temperature of 14 °C and fed a diet of 1 d old *Artemia* nauplii. The water was renewed and scyphistomae were fed 3 times a week. These scyphistomae made up the first acclimated generation (F_0).

Three weeks before acclimation, 75 well-fed, healthy scyphistomae with fully developed and extended tentacles were carefully removed from the wall of the kreisel tank with a Pasteur pipette, and each was transferred into a cylindrical plastic pot of 50 ml volume, filled with the original seawater from the kreisel tank. The 75 pots with individual scyphistomae were then moved into 3 temperature-controlled incubators (16 ± 0.5 °C), each containing 25 pots, and maintained in darkness. Dark conditions were chosen to minimise algae growth and mimic their natural habitat (Jarms et al., 2002; Raskoff et al., 2002). Scyphistomae were allowed to reattach to the plastic pot and acclimate to the new ambient conditions for 14 d.

Temperatures of 8, 12 and 16 °C were chosen for this study to imitate the mid-season thermal range that *A. aurita* scyphistomae may experience in their natural habitat. At 24 d prior to the experiment, 25 scyphistomae destined for the 8 °C treatment were slowly acclimated from 16 to 12 °C at the rate of 1.0 °C every 3 d. Once 12 °C had been reached, these and a second group of 25 scyphistomae were acclimated simultaneously at the same speed (i.e. 1.0 °C every 3 d), from 12 to 8 °C and from 16 to 12 °C, respectively, so that all scyphistomae arrived at their acclimated temperature of 16, 12 or 8 °C at the same time. The newly settled scyphistomae were the first acclimated generation (F_0), while their asexually reproduced buds made the first experimental generation (F_1) for transgenerational exposure.

Culture maintenance

The initial culture was set up with the original seawater. Thereafter, the water was renewed and scyphistomae were fed 3 times a week, according to the following protocol.

Seawater with debris, uneaten food and translucent biofilm were discarded. Particles settled on the bottom and biofilm adhering to the wall were re moved using a pipette. In normal conditions, a minimum of 65% of the water was changed, depending on the turbidity. Water for exchange was stored in 3 clean beakers kept in the 3 incubators and refilled after every cleaning, so that the correct temperature was maintained throughout the experiment. Salinity of the water was maintained at 31.5 by mixing 1 µm filtered natural seawater (water obtained from the National Oceanography Centre, Southampton, salinity ca. 33−35) and reverse osmosis water. After each water change, each scyphistoma was fed with 1 d old *Artemia* nauplii mixed with dried zooplankton (ZM Fish Food & Equipment, ZM-100, particle size 80−200 µm) to excess (based on the standard food portion in Hubot et al. 2017). This was intended to provide equal feeding in all treatments, avoiding food limitation at higher temperatures (Ma and Purcell, 2005). After the *Artemia* nauplii were added, the culture pots were maintained in darkness to ensure even distribution of food in the water. *Artemia* nauplii are positively phototactic and would normally assemble where light intensity is greatest, typically somewhere near the surface where the scyphistomae cannot capture them (Jarms et al., 2002). Handling time was kept to an absolute minimum.

Transgenerational exposure design

The acclimated generation (F_0) consisted of 3 temperature treatments (8, 12, 16 °C) with 25 replicates per treatment. The experimental generation (F_1) was also exposed to the 3 ecologically relevant temperatures of 8, 12 and 16 °C, capturing the full range experienced by polyp populations from southern England in late winter to late spring and early autumn to early winter, avoiding minimum winter and maximum summer temperatures. However, scyphistomae at each temperature had different histories of maternal temperature. The orthogonal combination of 3 maternal temperatures and 3 offspring temperatures resulted in 9 treatments in the F_1 generation in total (Figure 6.1). The 9 treatments are classified into 3 groups according to offspring temperature (i.e. X−8 °C group, X−12 °C group, X−16 °C group), with each group composed of scyphistomae from different origins. For example, the X−12 °C group is composed of 8 polyps that came from the F_0 generation exposed to 8 °C (i.e. 8−12 °C treatment), 8 polyps that came from the F_0 generation exposed to 12 °C (i.e. 12−12 °C treatment) and 8 polyps that came from the F_0 generation exposed to 16 °C (i.e. 16−12 °C treatment) (Figure 6.1). From the third week after temperature stabilization of the F_0 generation, every newly produced bud (F_1 generation) with fully developed tentacles was gently liberated from the parent scyphistoma with a pipette and transferred into a new pot containing water of the equivalent temperature and salinity. After 3 d of maintenance in the original incubator, the F_1 generation was then acclimated slowly to the test temperature at the same pace as their parents (1.0 °C every 3 d). During the acclimation phase of

the F_1 generation, 2 extra incubators were used for temperature transition. Eight individual polyps were cultivated for each treatment of F_1, resulting in 72 samples in total (Figure 6.1).

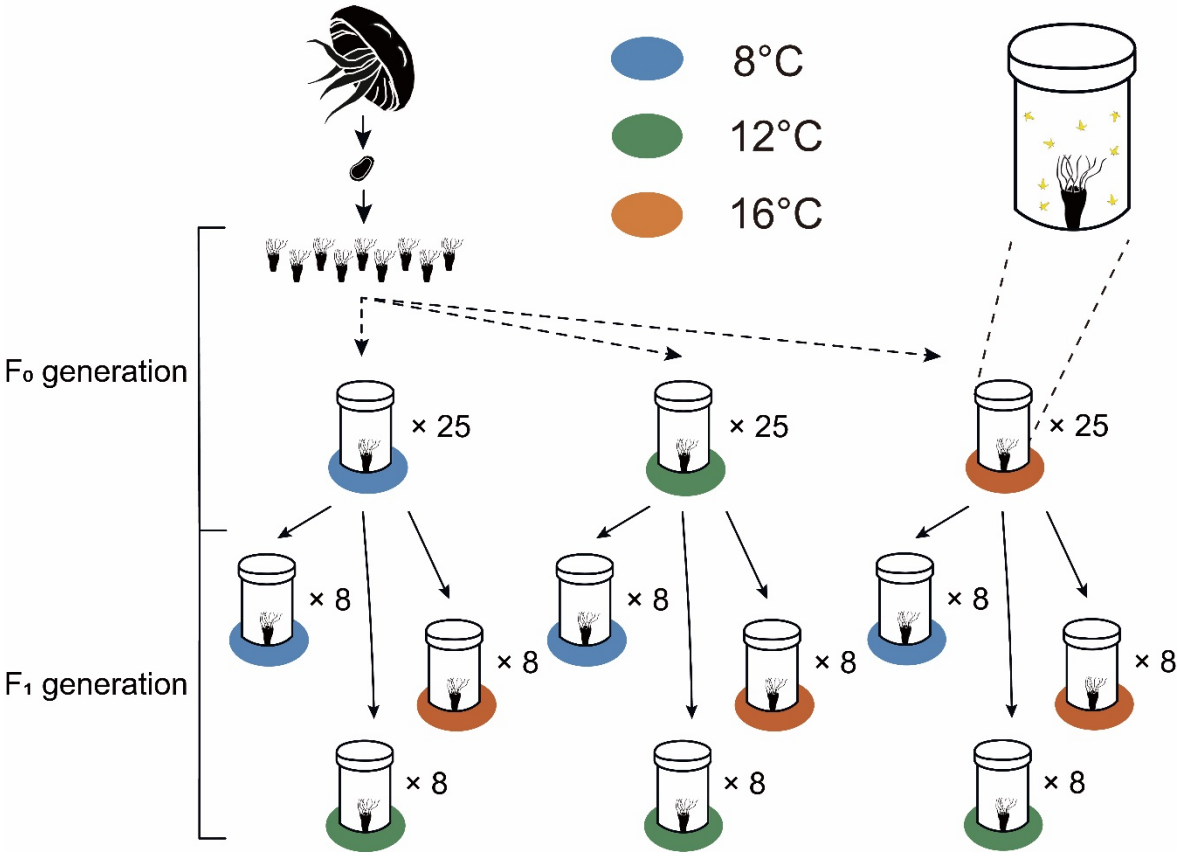

Figure C.1. Experimental design, consisting of 2 parts: a 45 d acclimation phase that exposed parental scyphistomae (F_0 generation) to 3 different temperatures, and an experimental (transgenerational exposure) phase, which subsequently exposed offspring scyphistoma (F_1 generation) from each origin to each temperature condition over 72 d. Each 50 ml plastic pot contained 1 scyphistoma

Data collection and statistical analyses

Immediately prior to each of the 3 times weekly cleaning events, each individual scyphistoma was checked for survival, attachment, fitness (colour and state of tentacles) and bud production. During data collection and cleaning, scyphistomae experienced some microscope light and indirect ceiling fluorescent room light, but this was kept to a minimum. The new buds or scyphistomae produced were identified and quantified following the description of Adler & Jarms (2009) and Schiariti et al. (2014). Briefly, direct buds, stolon buds and podocysts were identified. Fully developed buds were excised with a soft pipette from the pot to retain only the initial scyphistoma in the experimental pot. Buds with tentacles that were not yet fully developed were counted, but retained in the experimental pot until fully developed and a record was made to avoid repeated counts later. Podocysts were not removed.

Scyphistomae lost or killed by handling were removed from the initial scyphistoma numbers and their data were not included in the analyses. Survival in each treatment was evaluated as the proportion of scyphistomae that survived to the end of the experimental phase. Surviving scyphistomae included both healthy individuals and those that were unhealthy but still alive. Actively budding scyphistomae referred to individuals that produced at least 1 bud and survived to the end of the experiment (i.e. actively budding scyphistomae ≤ surviving scyphistomae). Budding rate was calculated from the total bud production per scyphistoma divided by the number of days that each scyphistoma survived and multiplied by the total days of the experiment to eliminate the effect of different survival times. The cumulative number of buds per scyphistoma was calculated as the addition of buds that each individual produced weekly. The proportion of stolon buds is the number of stolon buds divided by number of total buds. In summary, the following asexual-reproductive response variables were recorded: (1) observed asexual reproduction modes, (2) number of total reproductive products, (3) number of each type of reproductive product, (4) attachment or detachment of scyphistomae, (5) number of living scyphistomae, (6) days to produce first bud (podocysts excluded). All statistical analyses in this study were conducted using GraphPad Prism 8 statistical software. Kolmogorov-Smirnov tests were initially performed on all datasets to test for normality. For each experimental group (the F_0 generation itself as a group; see 'Transgenerational exposure design' for definition of the 3 groups in the F_1 generation) that passed assumptions of normality, parametric ANOVAs were performed; for those datasets that failed these assumptions, non-parametric Kruskal Wallis analyses were carried out. For parametric datasets, if assumptions of homogeneity of variances were accepted, multiple comparisons via Fisher's LSD method were performed following the ANOVA. Otherwise, Brown-Forsythe and Welch ANOVA tests were performed instead of 1-way ANOVA. Response variables (mean no. of direct buds scyphistoma^{-1}, mean no. of stolon buds scyphistoma^{-1}, mean no. of total buds scyphistoma^{-1} and mean proportion of stolon buds) were compared across temperature treatments for both the F_0 and F_1 generations. Additionally, we examined temperature effect on days to produce the first bud in the F_1 generation. A Kruskal Wallis test followed by multiple comparisons via an uncorrected Dunn's test was performed for all response variables in the F_0 generation and for most variables in the F_1 generation. However, 1-way ANOVA was applied to 'mean no. of direct buds scyphistoma^{-1}' and 'mean no. of total buds scyphistoma^{-1}' for the X−12 °C group, since the data set passed the normality test. For the X−8 °C group, there were insufficient live specimens in the 8−8 °C treatment regarding 'proportion of stolon buds' and 'days to produce the first bud'; thus, statistical analyses were only performed on the remaining 12−8 °C and 16−8 °C treatments. In this case, an unpaired 2-tailed t-test was applied to 'days to produce the first bud' where the data passed the test of normality, while a Mann-Whitney test was applied to 'mean proportion of stolon buds' where the data failed the test of normality. Two-

way ANOVA was used to examine the effects of maternal temperature, offspring temperature and their interactions on total number of buds produced scyphistoma^{-1} and days to produce first bud in the F_1 generation. Results were considered significant at $p < 0.05$ (Sokal & Rohlf 1995).

C.1.4 Results

Overall fitness and mode of reproduction

The stalk colour of polyps at all temperatures of both generations was a healthy orange. In the F_0 (parental) generation, there was 100% survival of *Aurelia aurita* scyphistomae in all 3 temperature treatments (Table 6.3). In the F_1 (offspring) generation, however, on the basis of a comparatively low sample number (8 individuals per treatment initially), scyphistomae survival ranged from 75−100%. The lowest survival in the F_1 generation, 75% (i.e. 6 out of 8), occurred in 4 treatments, of which the 'current' temperature was either 8 or 16 °C (Table 6.4). The overall high survival (100%, F_0 generation and ≥75%, F_1 generation) of *A. aurita* scyphistomae throughout the experiment suggests good tolerance to experimental conditions, which were well within their natural temperature range. The proportion of attached individuals of surviving scyphistomae was high (88−100%) in the F_0 generation (Table 6.3) and ranged between 50 and 100% in 9 treatments of the F_1 generation (Table 6.4). The lowest proportion (50%) of attached scyphistomae occurred in the 12−16 °C treatment. In the other 8 treatments of the F_1 generation, the attachment proportion was within a more reasonable range (83− 100%). Asexual reproduction occurred in both generations. No strobilation took place during the experimental period. Overall, 3 types of asexual reproduction modes were observed in this study: buds directly produced off the parental stalk (direct buds, Figure 6.2a), buds formed at the middle or end of the parental pedal stolon (stolon buds, Figure 6.2b) and cysts covered with a hard cuticle (podocysts, Figure 6.2c). Direct buds and stolon buds were produced in both generations. Podocyst formation was noted in all 3 treatments in the F_0 generation. Nevertheless, not every single scyphistoma in the F_0 generation produced podo cysts, and not every podocyst endured to the end of the experiment.

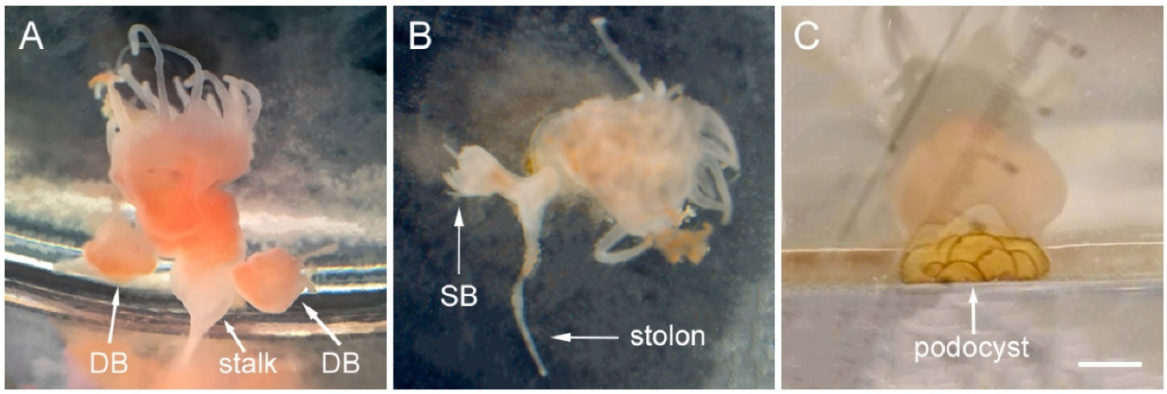

Figure C.2. Different asexual reproduction modes in *Aurelia aurita*: (a) typical direct buds (DB) formed at the pedal stalk; (b) stolon bud (SB) produced by the stolon; (c) a cluster of podocysts formed beneath the basal region of a scyphistoma. Scale bar: 1 mm

Table C.1. Summary of the results of temperature effects on *Aurelia aurita* scyphistomae in the F_0 generation during the 45 d experiment. The initial number of scyphistomae in each temperature treatment was 25. Standard error of the mean (Devbes et al.) is given in parentheses. Significantly different pairwise comparisons are indicated by different superscripts

Variable	**8 °C**	**12 °C**	**16 °C**
Surviving scyphistomae	25	25	25
Attached scyphistomae	22	23	25
Actively budding scyphistomae	20	16	22
Mean no. of total buds scyphistoma^{-1}	1.92 (0.29)^{a}	2.44 (0.44)^{a}	3.68 (0.42)^{b}
Mean no. of direct buds scyphistoma^{-1}	1.68 (0.26)^{a}	1.72 (0.33)^{a}	2.92 (0.35)^{b}
Mean no. of stolon buds scyphistoma^{-1}	0.24 (0.10)	0.72 (0.19)	0.76 (0.19)
Mean proportion of stolon buds	0.11 (0.05)^{a}	0.29 (0.07)^{b}	0.21 (0.05)^{ab}

Effects of temperature on asexual reproduction of the parental (F_0) generation

During the 45 d experiment, asexual reproduction did not occur in every surviving scyphistoma in the F_0 generation (see 'actively budding scyphistomae' in Table 6.3). A total of 158 direct buds were produced by 58 scyphistomae, and a total of 43 stolon buds were produced by 29 scyphistomae. Production of direct buds dominated asexual reproduction, accounting for 78.6% of total bud production (i.e. direct buds + stolon buds). The mean proportion of stolon buds

scyphistoma^{-1} was significantly affected by temperature (Kruskal-Wallis test, p = 0.0429), with a significantly higher proportion at 12 than at 8 °C (p = 0.0130). When standardized by days (i.e. number of buds scyphistoma^{-1} d^{-1}), reproduction rate of stolon buds at 12 °C changed from 0.005 buds scyphistoma^{-1} d^{-1} in the first 16 d to 0.022 buds scyphistoma^{-1} d^{-1} afterwards (Figure 6.3b). In 8 and 16 °C treatments, reproduction rate of stolon buds experienced no remarkable change. The cumulative number of direct buds and total buds in all 3 treatments increased almost linearly with time, indicating a relatively stable reproduction rate (Figure 6.3a,c).

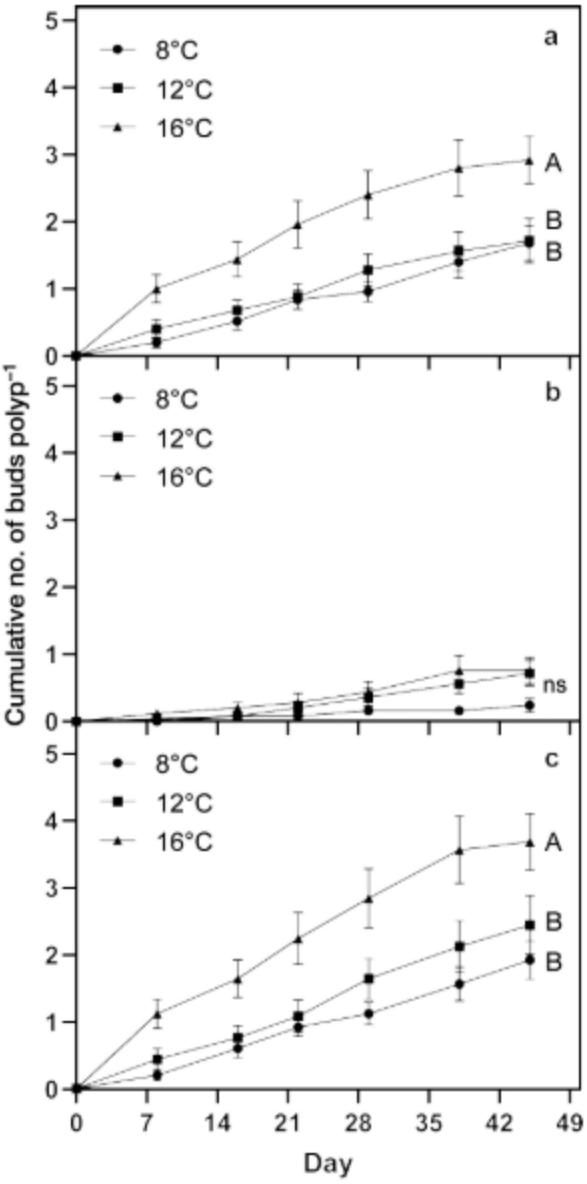

Figure C.3. F_0 generation. Time-series of cumulative number of (a) direct buds, (b) stolon buds and (c) total buds (direct + stolon) produced per polyp at each temperature (8, 12, 16 °C) during a 45 d experiment. Significant differences in Fisher LSD pairwise comparisons test following Kruskal Wallis test among 3 treatments are indicated by different letters; ns: not significant. Error bars show SEM

At the end of the 45 d experiment, the greatest numbers of direct, stolon and total buds scyphistoma^{-1} were produced at 16 °C and the fewest were produced at 8 °C (Figure 6.3). Effects of temperature were significant on the number of direct buds scyphistoma^{-1} (Kruskal Wallis test, p = 0.0211). Significantly greater numbers of direct buds scyphistoma^{-1} were produced at 16 than at 8 °C (p = 0.0162) and 12 °C (p = 0.0160), while in 8 and 12 °C treatments, similar numbers of direct buds scyphistoma^{-1} were produced (p > 0.05). There were no significant differences in number of stolon buds scyphistoma^{-1} at the 3 temperatures (Kruskal Wallis test, p > 0.05). Temperature effects on the number of total buds scyphistoma^{-1} were significant (Kruskal Wallis test, p = 0.0117), with greater production of total buds at 16 than at 8 °C (p = 0.0039) and at 12 °C (p = 0.0375). The number of days to produce the first bud was similar across the 3 treatments, ranging from 8 to 11.

Effects of temperature on asexual reproduction of offspring (F_1) generation

During the 72 d exposure process, 46 out of 62 surviving scyphistomae reproduced by budding in the F_1 generation (see 'actively budding scyphistomae' in Table 6.4). A total of 68 direct buds were produced by 40 scyphistomae, and a total of 27 stolon buds were produced by 20 scyphistomae. Thus, production of direct buds dominated asexual reproduction, accounting for 71.6% of total bud production (i.e. direct buds + stolon buds). In the 8−8 °C treatment, only 1 scyphistoma produced buds (1 direct bud and 1 stolon bud) (Table 6.4). In the other 8 treatments, the mean proportion of stolon buds was <50%, with the highest proportion (46.7%) in the 8−12 °C treatment and lowest (15.6%) in the 8−16 °C treatment. Preliminary analysis revealed that temperature exerted significantly different effects on number of direct buds scyphistoma^{-1} in X−12 °C treatments (1-way ANOVA, p = 0.0146) and X−16 °C treatments (Kruskal-Wallis test, p = 0.0015), but not on the number of stolon buds scyphistoma^{-1} nor proportion of stolon buds produced in the F_1 generation (Table 6.6 in on p.137). Direct buds and stolon buds are therefore not considered separately in the following sections; total buds (direct buds + stolon buds) are shown instead.

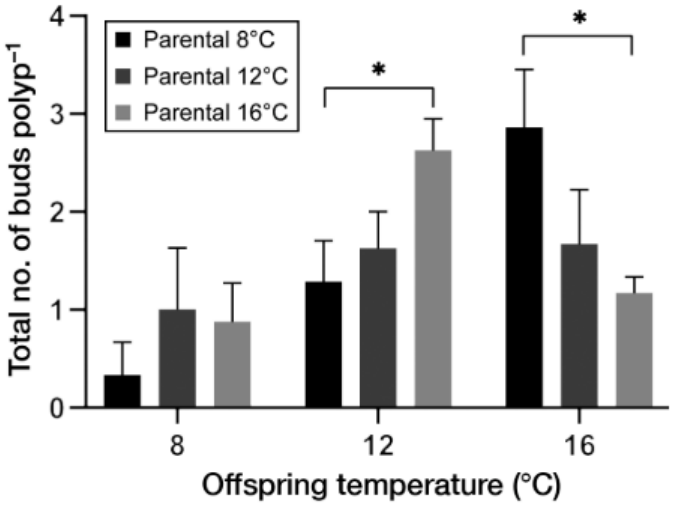

Figure C.4. F_1 generation. Overall number of total buds produced per polyp at 3 offspring temperatures (8, 12, 16 °C) with different histories of parental temperatures (8, 12, 16 °C) at the end of a 72 d experiment. Significant differences in Fisher LSD pairwise comparisons test following a 1-way ANOVA, or an uncorrected Dunn's test following a Kruskal Wallis test, among each treatment group with the same offspring temperature are indicated by asterisks (*$p < 0.05$). Error bars show SEM

Table C.2. Summary of effects of maternal temperature on *Aurelia aurita* scyphistomae of the F_1 generation during the 72 d experiment (see Figure 6.1 for details of the experimental set-up). The initial number of scyphistomae in each treatment was 8. Standard error of the mean (Devbes et al.) is given in parentheses (an asterisk [*] indicates that the 8−8 °C treatment had only 1 actively budding scyphistoma, so a calculation of SEM was inapplicable). Significantly different pairwise comparisons are indicated by different superscripts

Variable	Offspring (F_1) temperature (°C)	Parental (F_0) temperature (°C)		
		8	12	16
Surviving scyphistomae	8	6/8	6/8	8/8
	12	7/8	8/8	8/8
	16	7/8	6/8	6/8
Attached scyphistomae	8	5/6	6/6	8/8
	12	7/7	8/8	7/8
	16	6/7	3/6	6/6
	8	1/6*	3/6	4/8

Variable	Offspring (F_1) temperature (°C)	Parental (F_0) temperature (°C)		
		8	12	16
Actively budding	12	5/7	7/8	8/8
	16	7/7	5/6	6/6
Mean no. of total	8	0.33 (0.33)	1.00 (0.63)	0.88 (0.40)
buds	12	1.29 (0.42)^{b}	1.63 (0.38)^{ab}	2.63 (0.32)^{a}
	16	2.86 (0.59)^{a}	1.67 (0.56)^{ab}	1.17 (0.17)^{b}
Mean no. of direct	8	0.17 (0.17)	0.50 (0.22)	0.63 (0.38)
buds scyphistoma^{-1}	12	0.71 (0.29)^{b}	1.25 (0.37)^{b}	2.38 (0.42)^{a}
	16	2.14 (0.26)^{a}	1.00 (0.26)^{b}	0.67 (0.21)^{b}
Mean no. of stolon	8	0.17 (0.17)	0.50 (0.50)	0.25 (0.16)
buds scyphistoma^{-1}	12	0.57 (0.20)	0.38 (0.26)	0.25 (0.16)
	16	0.71 (0.42)	0.67 (0.49)	0.50 (0.22)
Mean proportion of stolon buds	8	0.50*	0.25 (0.25)	0.38 (0.24)
	12	0.47 (0.16)	0.24 (0.16)	0.07 (0.14)
	16	0.16 (0.07)	0.25 (0.16)	0.33 (0.14)
No. of buds Scyphistoma^{-1} d^{-1}	8	0.037	0.063	0.038
	12	0.043	0.071	0.164
	16	0.044	0.027	0.051

When standardized by days (Table 6.4), the 12−8 °C treatment displayed the greatest reproduction rate (0.0625 buds scyphistoma^{-1} d^{-1}) among X−8 °C treatments. For X−12 °C treatments, the highest budding rate occurred in the 16−12 °C treatment (0.164 buds scyphistoma^{-1} d^{-1}). Among X−16 °C treatments, the 8−16 °C treatment displayed a budding rate of 0.026 buds scyphistoma^{-1} d^{-1} between Days 7 and 62, before increasing sharply to 0.143 buds scyphistoma^{-1} d^{-1} during the last 10 d of the experiment (Figure 6.7).

At the end of the 72 d experiment, the X−8 °C group produced similar numbers of total buds (Kruskal Wallis test, $p > 0.05$) (Table 6.4, Figure 6.4), ranging from 0.33 bud scyphistoma^{-1} (8−8 °C treatment) to 1 bud scyphistoma^{-1} (12−8 °C treatment). For scyphistomae in X−12 °C treatments, parental temperature exerted significant effects on the number of total buds produced per polyp (1-way ANOVA, $p = 0.049$). The 16−12 °C treatment produced significantly more total buds than the 8−12 °C treatment ($p = 0.021$). The number of total buds in the 12−12 °C treatment did not

differ significantly from those in the 8−12 or 16−12 °C treatments (both $p > 0.05$) (Table 6.4, Figure 6.4). The number of total buds produced in X−16 °C treatments was significantly affected by parental temperature (Kruskal Wallis test, $p = 0.0394$). Scyphistomae in the 8−16 °C treatment produced significantly more total buds than the 16−16 °C treatment ($p = 0.0156$), although the number of total buds did not differ significantly between 8−16 °C and 12−16 °C treatments ($p > 0.05$), nor between 12−16 °C and 16−16 °C treatments (both $p > 0.05$) (Table 6.4, Figure 6.4).

The number of days to produce the first bud was similar among treatments at an F_1 temperature of 8 °C (unpaired 2-tailed t-test, $p > 0.05$) (Figure 6.5). For X−12 °C treatments, the effects of parental temperature on timing of budding were significant (Kruskal Wallis test, $p = 0.0306$); the 8−12 °C treatment took significantly less time (55.2 d) to produce the first bud than the 12−12 °C treatment (63.6 d) ($p = 0.0104$). For treatments at 16 °C, the timing of budding differed significantly with different parental temperature (Kruskal Wallis test, $p < 0.001$). Scyphistomae in the 16−16 °C treatment started budding significantly later (58.2 d) than those in the 8−16 °C (23.9 d) and 12−16 °C (32.4 d) treatments ($p = 0.001$ and 0.033, respectively).

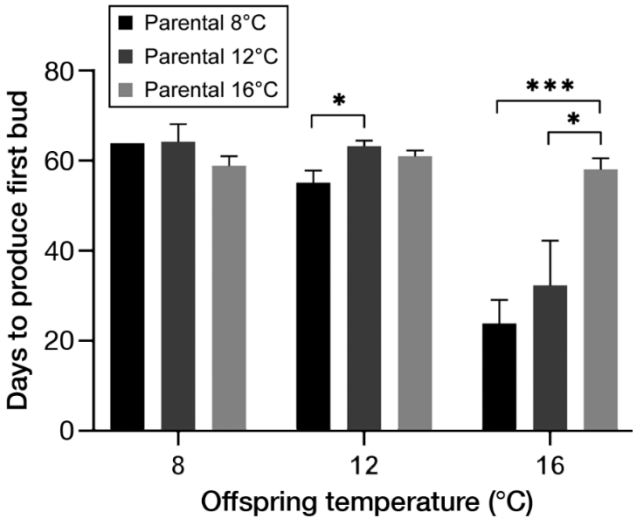

Figure C.5. F_1 generation. Average number of days taken by every scyphistoma to produce the first bud at 3 offspring temperatures (8, 12, 16 °C) with different parental temperature histories (8, 12, 16 °C) during the 72 d experiment. *$p < 0.05$, ***$p < 0.001$. Error bars show SEM

Two-way ANOVA showed the effects of parental temperature, offspring temperature and their interactions on 2 reproduction variables in the F_1 generation. We found that after 72 d of transgenerational temperature exposure, the interaction between parental temperature and offspring temperature was abundantly clear on the number of total buds produced per scyphistoma (interaction term $p < 0.05$, Table 6.5), with a significant effect of offspring temperature ($p = 0.003$) and no significant effect of parental temperature ($p = 0.941$).

Concurrently, the parental temperature × offspring temperature interaction was significant on the number of days to produce first bud (interaction term $p < 0.05$, Table 6.5), with a significant effect of both parental and offspring temperature ($p = 0.0493$ and $p < 0.0001$, respectively).

Table C.3. Results of 2-way ANOVA for response variables of the F_1 generation showing effects of parental temperature, offspring temperature and their interaction. SS (Type III): adjusted (Type III) sums-of-squares; MS: mean square

Source	df	SS (Type III)	MS	*F*	p
Mean no. of total buds scyphistoma^{-1}					
Parental temperature (°C)	2	0.1609	0.021	0.0606	0.9413
Offspring temperature (°C)	2	17.16	0.092	6.459	0.0031
Interaction (parental temp x offspring temp)	4	18.41	0.002	3.464	0.0138
Residual	53	70.41	0.002		
Days to produce first bud					
Parental temperature (°C)	2	695.2	347.6	3.284	0.0493
Offspring temperature (°C)	2	4934	2467	23.31	<0.0001
Interaction (parental temp x offspring temp)	4	2086	521.4	4.926	0.0029
Residual	35	3705	105.8		

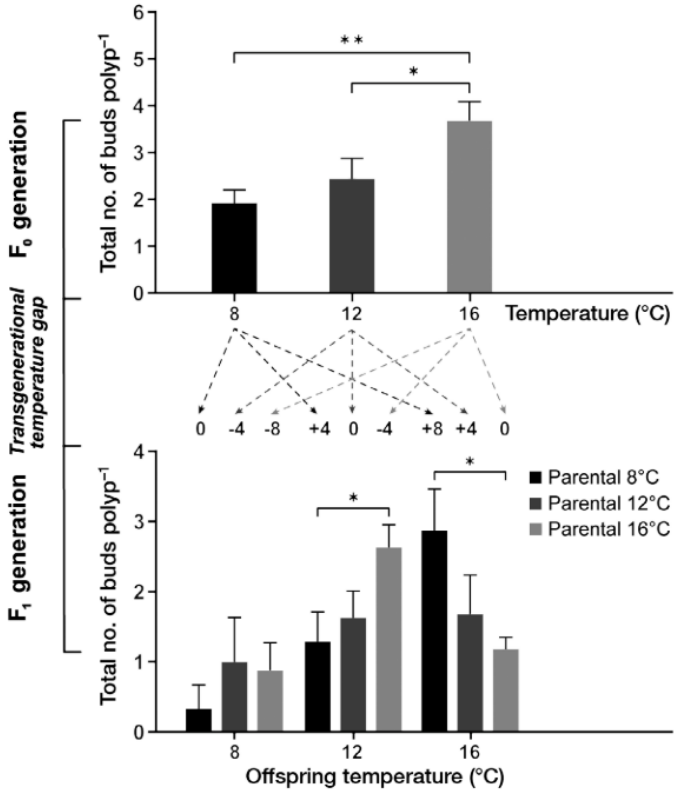

Figure C.6. F_0 and F_1 generations. Overall number of total buds produced per polyp in both parental and offspring generations. The thermal origin and fate of scyphistoma in the F_1 generation are indicated by dashed arrows. Above each corresponding column, the transgenerational temperature gap experienced by scyphistomae in each treatment of the F_1 generation is indicated by 0, +4/−4 or +8/−8. Significant differences in Fisher LSD pairwise comparisons test following a 1-way ANOVA, or an uncorrected Dunn's test following a Kruskal Wallis test, among each comparable dataset are indicated by asterisks (*$p < 0.05$, **$p < 0.01$). Error bars show SEM

C.1.5 Discussion

Asexual reproduction patterns

Among the diverse modes of asexual reproduction of *Aurelia aurita* scyphistomae, e.g. direct buds, stolon buds, planuloid buds, longitudinal fission, podocyst formation, free-swimming propagules and regeneration (Vagelli, 2007; Arai, 2009; Schiariti et al., 2014), only direct buds, stolon buds and podocysts were observed in our study. Production of direct buds dominated asexual reproduction in both parental and offspring generations (78.6 and 71.6% of total buds, respectively). A similar finding was reported by Han & Uye (2010), whereby direct budding accounted for 94% of asexual reproduction displayed by *A. coerulea* scyphistomae in a laboratory culture. Additionally, podocysts, with the ability to protect the population against predatory

pressure and ensure survival during adverse conditions, can potentially develop into new scyphistomae by excystment (Thein et al., 2012; Schiariti et al., 2014). Unfavourable environmental factors related to temperature and food availability have been suggested to cause podocyst production in scyphozoan jellyfish polyps (Arai, 2009; Thein et al., 2012), but it is unclear why podocysts formed in the current study.

Evidence of transgenerational acclimation in the F_1 generation

Under the designed temperature conditions, there were 3 major scenarios encountered by scyphistomae of the F_1 generation: constant temperature, transgenerational warming (+4, +8 °C) and transgenerational cooling (−4, −8 °C) (Figure 6.6). Comparisons were drawn among treatments with the same F_1 temperature to evaluate the effects of parental temperature and the transgenerational temperature gap on reproductive output and timing in the F_1 generation. Hence, transgenerational cooling with constant temperature is discussed for X−8 °C treatments, while transgenerational warming with constant temperature is discussed in X−16 °C treatments. The X−12 °C treatments include all encountered scenarios.

For X−8 °C treatments, scyphistomae started budding at similar times and produced similar number of total buds, independent of the thermal history of the parental generation. In addition, the proportions of actively budding scyphistomae to surviving scyphistomae were <50%. It seems that cooling down to 8 °C or being maintained at 8 °C caused significant inhibition of bud production and budding onset in *A. aurita* scyphistomae. As 8 °C is near the lower limit of the thermal window of *A. aurita* in Horsea Lake, stagnation of asexual reproduction was predicted, and is consistent with observations from other studies. For naturally occurring scyphistomae colonies, asexual bud production may decline to nearly zero during the coldest part of the year (Willcox et al., 2007). In a laboratory test, scyphistomae cultured at 14 °C, whose medusae were derived from the Red Sea (temperature range 20.9− 26.4 °C), showed markedly lower budding rates than at warmer temperatures (Pascual et al., 2015).

Among X−12 °C treatments, scyphistomae whose parents were exposed to a warmer (16 °C) temperature had significantly greater output (number of buds polyp^{-1}) compared with scyphistomae whose parents were from cold (8 °C) conditions. The early- to mid-spring temperature in Horsea Lake is ~12 °C when budding commences in natural populations (Lucas et al., 2012). We speculate that scyphistomae produced by parents from the warmer temperature showed greater reproductive potential than those from ‘cold parents’ at the intermediate temperature of 12 °C, suggesting a transgenerational effect.

In X−16 °C treatments, a significant difference occurred in the production of total buds between the +8 °C (8− 16 °C) and constant temperature (16− 16 °C) treatments (2.86 and 1.17 buds scyphistoma^{-1}, respectively), indicating enhancement of reproductive output in the F_1 generation by transgenerational warming of +8 °C. However, there was a lack of significant difference observed between treatments of +4 °C warming (12−16 and 8−12 °C) versus corresponding constant (16−16 °C and 12− 12 °C) temperatures. As such, the amplitude of environmental fluctuation influences the parental effect (Mousseau and Fox, 1998): the greater the environmental fluctuation, the greater the selection for an inherited environmental effect (Rossiter, 1996). These hypotheses may explain the results in +4 °C treatments, whereby transgenerational warming of 4 °C may not be great enough to induce statistically significant effects on reproductive output within the experimental temperature range tested. Regarding the time taken to produce the first bud, +4/+8 °C treatments started budding significantly earlier than treatments with constant temperatures. This indicates that a positive across-generation temperature change (i.e. transgenerational warming) triggered an earlier budding event in offspring scyphistomae within the experimental thermal range. In a similar manner, egg hatching of the copepod *Acartia* sp. has been shown to accelerate with warmer egg production temperature (Vehmaa et al., 2012). The acclimation process experienced by the parental (F_0) generation (from settlement temperature of 16 °C to experimental temperatures of 8, 12 and 16 °C) may be regarded as being equivalent to a short-term exposure phase, with 16 °C as the control. The timeline of the offspring (F_1) generation consisted of a preparation phase and a reproductive phase, so the scyphistomae whose parental temperature was 16 °C experienced a somewhat similar process as the F_0 generation. Hence, we might be able to compare the trends, but not the exact values, of budding output and timing, as displayed by both groups. In the F_0 generation, the greatest budding output occurred at 16 °C (3.68 buds scyphistoma^{-1}, $p = 0.0117$) (Figure 6.3), while in the F_1 generation, the 16−12 °C treatment produced significantly more buds (2.63 buds scyphistoma^{-1}) than the 16−8 °C treatment (0.88 bud scyphistoma^{-1}) ($p = 0.0028$) and 16−16 °C treatment (1.17 buds scyphistoma^{-1}) ($p = 0.0279$) (Figure 6.8). The start-time of budding was similar in both the F_0 generation and 16−X °C treatments in the F_1 generation. This suggests that differences in reproductive parameters of the F_1 generation were caused by the thermal transgenerational effects, not the independent influence of the offspring generation (short-term exposure).

Ecological implications

In the current study, the gap between parental and offspring temperature can be deemed as rapid environmental fluctuations (Paenke et al., 2007). In this case, a mismatch between the temperature environment of parents and offspring can elicit a phenotypic response by individuals

in the offspring generation (i.e. reproductive traits in this study) (LaMontagne and McCauley, 2001). TGP, in particular thermal TGP, has been investigated for a variety of traits and taxa. The walking speed of *Drosophila melanogaster* was greater for offspring whose parents were reared at higher temperatures, independent of the offspring thermal environment (Gilchrist and Huey, 2001). For the marine bryozoan *Bugula neritina* that reproduces by asexual budding, offspring from parents kept in warmer water had smaller and more variable size, with increased dispersal potential and higher metamorphic success than those from 'cooler' parents (Burgess and Marshall, 2011). Parthenogenetic *Daphnia magna* females held at a higher temperature produced offspring with greater resistance to parasite infection (Garbutt et al., 2014) and with enhanced tolerance to toxic *Microcystis* (Lyu et al., 2017). In addition to thermal TGP, a growing body of research has investigated the transgenerational consequences of ocean acidification (Parker et al., 2012), hypoxia (Wang et al., 2016), salinity stress (Jeremias et al., 2018) and contaminants (Schwindt, 2015), with increasing evidence elucidating the molecular mechanisms (e.g. DNA methylation) (Wang et al., 2016; Ryu et al., 2018).

The most common scenario of transgenerational thermal acclimation for naturally occurring scyphistomae populations may be the seasonal temperature warming from spring to summer. With potentially greater temperature elevation under future climate conditions, the reproductive potential of offspring scyphistomae is predicted to increase accordingly. When faced with seasonal cooling, the reproductive potential from a previous 'warm' history would persist until the next reproduction event. If the same scyphistomae colonies survive to the next year, the reproductive potential of their offspring might be further enhanced, despite the common year-to-year variability in medusae abundance in natural habitats (Lucas et al., 2012). TGP may favour potentially invasive species such as *Aurelia* jellyfish by enhancing the ability of the species to acclimate to conditions in a new environment and boosting its invasion success via increased fitness and survival of offspring (Lenz et al., 2011; Podbielski et al., 2016). Many of the bloom-forming jellyfish species, including *Aurelia* spp., are characterized by good tolerance to a wide range of abiotic conditions (Lucas, 2001), coupled with flexible reproductive traits as revealed by the present study. Many of the most damaging jellyfish blooms have been caused by nonindigenous species (Xian et al., 2005) that cause significant ecosystem disruption and economic loss in their new habitat (Manzari et al., 2015) (but see *Pelagia noctiluca* as an example of a native species causing widespread harm in the NE Atlantic, Doyle, 2008) and Mediterranean (Canepa et al., 2014). Earlier budding initiation in *A. aurita* scyphistomae is likely to reduce the risk of being buried and the negative effects of intraspecific competition (Schiariti et al., 2015), thus gaining a competitive advantage for limited resources (Vehmaa et al., 2012). Further, earlier budding and greater budding output will likely enhance the potential for rapid colonization and

expansion into new habitats (Schiariti et al., 2015), setting the stage for subsequent large blooms when favourable conditions prevail (Purcell et al., 2007). Transgenerational acclimation may also provide a good opportunity to study local adaptation (Sanford and Kelly, 2011) and bloom dynamics of introduced jellyfish species (Abboud et al., 2018).

Worldwide, rising temperatures pose serious threats to marine organisms and ecosystems (Doney et al., 2012). Shifts in the timing of life history events (Parmesan, 2007) and geographic distributions (Zhang et al., 2020) that are associated with these globally fundamental changes have already been observed in a variety of marine species (reviewed by Poloczanska et al. 2013). Global warming may facilitate a shift to dominance by non-native species in 2 ways: giving introduced species an earlier start (consistent with our finding of the advanced budding initiation), and increasing the magnitude of their growth (consistent with our finding of the enhanced reproduction output) and recruitment relative to natives(Stachowicz et al., 2002).

Though barely explored in jellyfish, transgenerational effects are prevalent in other aquatic organisms such as a marine bryozoan (Burgess and Marshall, 2011), sheepshead minnows (Salinas and Munch, 2012), a marine polychaete (Massamba-N'Siala et al., 2014) and symbiotic Hydra (Ye et al., 2019). Klein et al. (2017) observed that pre-exposure of mother scyphistomae of *Alatina alata* (Cnidaria, Cubozoa) to elevated temperature could partially mitigate the negative effects of elevated temperature and reduced pH on reproduction of daughter scyphistomae, suggesting interactions between different climate change stressors (Byrne and Przeslawski, 2013). For marine organisms, transgenerational acclimation is likely to help them persist in a rapidly changing ocean (Munday et al., 2013), by partially or fully ameliorating negative effects of warming, acidification and hypoxia (Munday, 2014). In the face of challenges raised by global climate change, the ability to match the phenotype of offspring to changes in ambient environment may be particularly important. Assessment of the (transgenerational) acclimation potential to environmental stress may also provide an understanding of how abiotic factors affect the distribution of marine species (Munday et al., 2013).

Despite a growing number of studies on thermal transgenerational effects, most studies have addressed only ‘warming’, either mild heat (Groot et al., 2017) or heatwaves (Sales et al., 2018) in climate-related scenarios, with less attention on ‘cooling’, which occurs seasonally in temperate and high-latitude environments. Our study ind icated that, when the destination of transgenerational temperature fluctuation (i.e. offspring temperature) was near the lower tolerance limit of the species, the influence of offspring temperature outweighed that of transgenerational effects.

C.1.6 Conclusions

The bloom-forming *Aurelia* jellyfish is a classic model genus in jellyfish bloom research. We have shown that, provided with a transgenerational temperature change, parent scyphistomae modify the reproductive response (budding output and timing) in their offspring, suggesting transgenerational acclimation in the asexual life-stage of *A. aurita*. In summary, the present study demonstrates that, within the experimental temperature range tested,

(1) Offspring from high- (16 °C) and low- (8 °C) temperature parents displayed the best reproductive potential at medium (12 °C) and high (16 °C) temperatures, respectively.

(2) Offspring from low-temperature (8 °C) parents showed the greatest advantage in an early start of re production event at medium (12 °C) and high (16 °C) temperatures, respectively.

(3) Asexual reproduction of offspring from parents at high (16 °C), medium (12 °C) and low (8 °C) temperatures was suppressed intensely at low (8 °C) temperature.

Unlike most previous studies conducted on a single/unknown generation, the merits of our experiment were the orthogonal design of 3 parental temperatures × 3 offspring temperatures, providing novel in sights for transgenerational acclimation in scyphistomae. Though often underestimated, the gelatinous plankton (including jellyfish) have been shown to be important members of marine pelagic food webs, particularly when present in large numbers. Thus, the magnitude and timing of their reproduction can have considerable ecological consequences on the whole ecosystem.

C.1.7 Additional Information

Table C.4. F_1 generation. Analysis of reproductive parameters in response to the same offspring temperature with different maternal temperature histories during 72 d. One-way ANOVA followed by Fisher's LSD test, or a Kruskal-Wallis test followed by uncorrected Dunn's test, was performed based on normality test. PT: parental temperature; OT: offspring temperature. Significant results ($p < 0.05$) are highlighted in bold

	PT-OT (°C)	p	Analysis method used
No. of direct buds produced	X-8	0.5728	Kruskal Wallis test
	X-12	**0.0146**	One-way ANOVA
	X-16	**0.0015**	Kruskal Wallis test

	PT-OT (°C)	p	Analysis method used
No. of stolon buds produced	X-8	>0.9999	Kruskal Wallis test
	X-12	0.4330	Kruskal Wallis test
	X-16	0.9861	Kruskal Wallis test
Proportion of stolon buds	X-8	0.8286	Mann Whitney test
	X-12	0.297	Kruskal Wallis test
	X-16	0.6196	Kruskal Wallis test

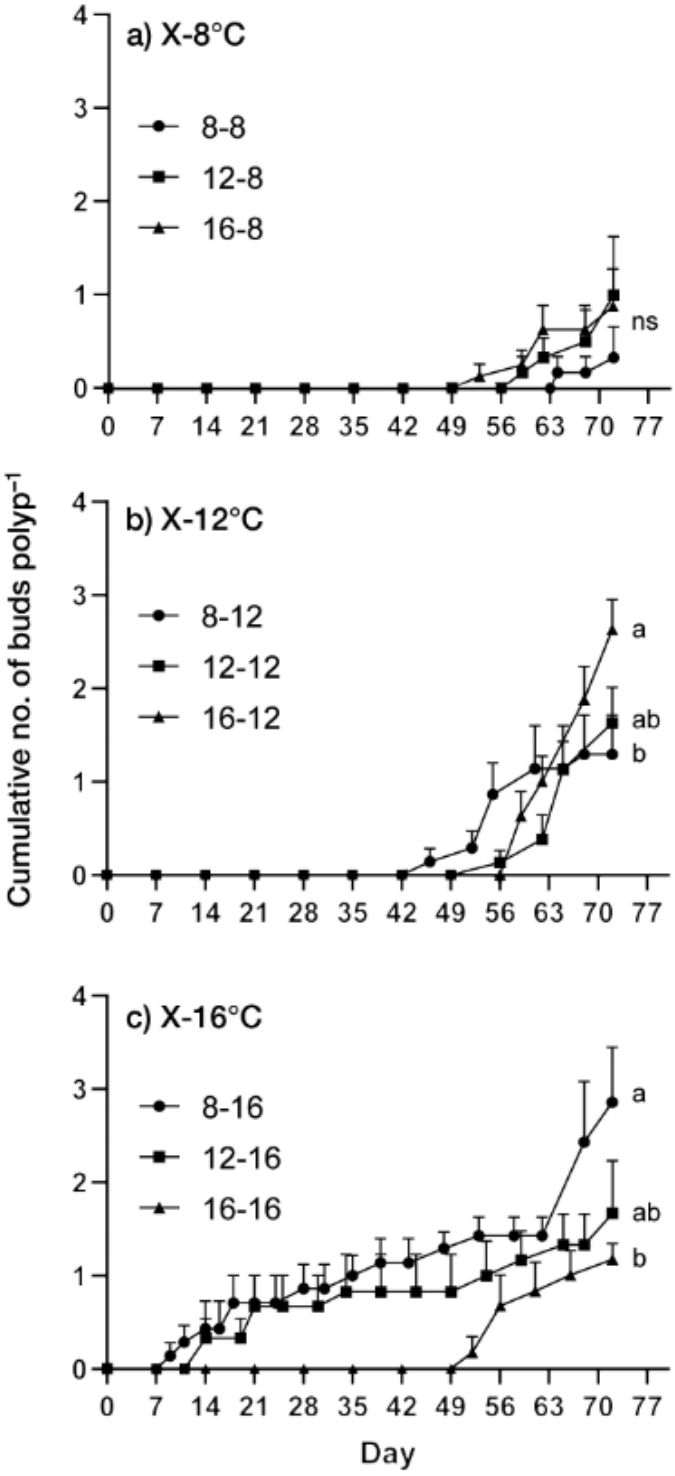

Figure C.7. F_1 generation. Time-series of cumulative number of total buds produced per polyp at each temperature (8, 12 and 16 °C), for offspring whose mothers had different exposure history (8, 12, 16 °C), during a 72 d experiment. Differences in Fisher LSD pairwise comparisons or uncorrected Dunn's test are indicated by different letters. Error bars show SEM

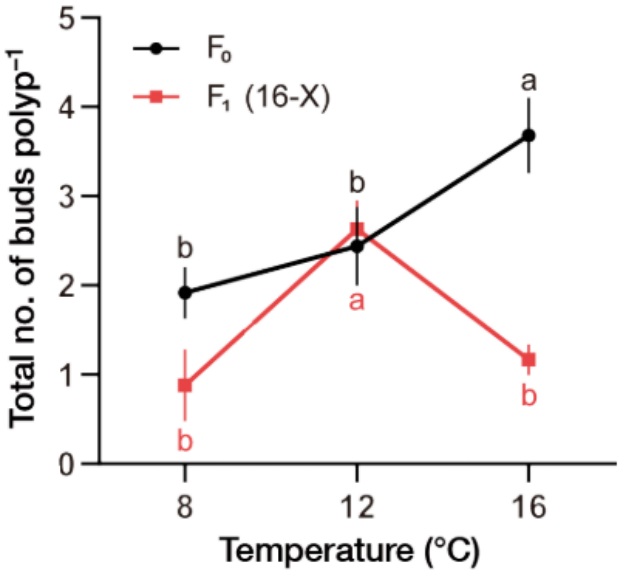

Figure C.8. F_0 generation and F_1 generation (16−X °C treatments). The number of total buds produced per polyp at each temperature (8, 12 and 16 °C), at the end of the 45 d experiment for the F_0 generation (black circles) and at the end of the 72 d experiment for the F_1 generation (red squares). Differences in uncorrected Dunn's test following the Kruskal Wallis test are indicated by different letters. Error bars show SEM

C.2 Molecular analysis

Total RNA was extracted from *Aurelia aurita* polyps using an established procedure: tissue was minced in a buffer containing proteinase K in the presence of a ribonuclease inhibitor (Rnasin). Following incubation at 55°C for 10 minutes, RNA was isolated by acidic phenol– chloroform–isoamyl alcohol extraction and subsequent isopropanol precipitation. RNA pellets were solubilized in RNase-free water and immediately.

Fifty frozen *Aurelia aurita* polyp samples stored at -80°C immediately after collection were sent to Source Bioscience for extraction of RNA and a qPCR gene expression microarray for relative quantification.

Table C.5. Sample list for samples sent for RNA extraction and analysis

Sample	n
15	3
17	3

19	3
15>15	3
15>17	3
15>19	3
17>15	3
17>17	3
19>15	3
19>19	3
15>15>15	4
15>15>19	3
15>17>19	3
15>19>19	3
19>15>15	3
19>19>19	4

Using a Nanodrop ND-1000 Spectrometer Analysis, the 260/280 and 260/230 ratio were analysed. Most samples indicated possible protein contamination, and some indicated possible contamination with organic solvents (Table 6.8). Contaminants in the samples can severely affect the processes involved in certain analysis applications (such as microarrays). The samples yielded generally low results, as expected for jellyfish polyps, and were input into the cDNA conversion reaction at 0.42ng/µl to generate sufficient amount of cDNA for the qPCR reaction. The endogenous control was ß-actin (Schroth et al., 2005) and the target gene was the heat shock protein Hsp70.

Table C.6. Concentration and sample quality: Nanodrop ND-1000 Spectrometer Analysis

Sample ID	**Conc. (ng/µl)**	**260/280**	**260/230**	**Yield (ng)**
15	12.05	1.97	0.27	168.7
15	0.58	1.3	0.05	8.12
15	20.74	1.95	0.56	290.36
17	0.92	2.11	0.01	12.88
17	0.67	7.79	0.02	9.38

Sample ID	Conc. (ng/µl)	260/280	260/230	Yield (ng)
17	8.29	1.63	0.27	116.06
19	0.7	1.2	0.07	9.8
19	1.23	1.49	0.12	17.22
19	0.61	0.85	0.04	8.54
15-15	7.84	1.81	0.22	109.76
15-15	5.1	1.79	0.24	71.4
15-15	7.79	1.88	0.25	109.06
15-17	7.45	1.58	0.07	104.3
15-17	0.83	1.41	0.06	11.62
15-17	6.59	1.56	0.36	92.26
15-19	1.91	1.26	0.04	26.74
15-19	0.19	0.38	0.01	2.66
15-19	2.47	1.25	0.04	34.58
17-15	10.39	1.75	0.32	145.46
17-15	4.77	1.69	0.1	66.78
17-15	10.23	1.77	0.21	143.22
17-17	0.42	2.29	0	5.88
17-17	7.48	1.88	0.08	104.72
17-17	4.61	1.48	0.06	64.54
19-15	5.4	1.7	0.07	75.6
19-15	7.17	1.58	0.37	100.38
19-15	5.26	1.96	0.07	73.64
19-19	1.71	1.26	0.01	23.94
19-19	6.56	1.86	0.09	91.84
19-19	7.31	1.6	0.05	102.34
15-15-15	3.68	1.31	0.04	51.52
15-15-15	3.08	1.28	0.08	43.12
15-15-15	1.21	3.03	0.05	16.94
15-15-15	3.6	1.33	0.1	50.4

Sample ID	Conc. (ng/µl)	260/280	260/230	Yield (ng)
15-15-19	5.66	1.43	0.16	79.24
15-15-19	1.3	-27.42	0.01	18.2
15-15-19	2.86	1.12	0.14	40.04
15-17-19	2.02	1.07	0.05	28.28
15-17-19	2.9	1.33	0.07	40.6
15-17-19	2.17	1.78	0.03	30.38
15-19-19	3.12	1.52	0.05	43.68
15-19-19	2.21	1.7	0.02	30.94
15-19-19	3.02	1.8	0.08	42.28
19-15-15	8.7	1.54	0.05	121.8
19-15-15	1.15	1.49	0.11	16.1
19-15-15	1.15	1.34	0.06	16.1
19-19-19	5.01	1.34	0.04	70.14
19-19-19	2.59	1.8	0.03	36.26
19-19-19	2.72	1.03	0.09	38.08
19-19-19	3.06	1.79	0.05	42.84

qPCR results indicated that only 23/50 samples amplified, and within those all three technical replicated did not always amplify. As well as this, no samples amplified before ~ 40 PCR cycles, indicating that the data was not of a sufficient quality. We include this information in the appendices to support any future research that may seek to analyse gene expression in *Aurelia aurita* and other jellyfish

Appendix D Supplementary information for Chapter 5

Table D.1. Cumulative number of directly budded polyps produced in each treatment across the experiment (n=10). Column headers refer to the treatment (i.e. 2W4C = replicates were held for 2 weeks at 4 °C)

DAY	2W4C	2W7C	2W10C	4W4C	4W7C	4W10C	6W4C	6W7C	6W10C	8W4C	8W7C	8W10C	4C	7C	10C	13C
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0
14	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1
21	1	2	0	0	0	2	0	0	0	0	0	1	0	0	0	4
28	1	2	0	0	0	2	0	0	1	0	0	1	0	0	1	5
35	2	3	1	0	0	2	0	0	1	0	0	2	0	0	4	6
42	2	4	1	0	0	2	0	0	1	0	0	2	1	0	4	7
49				1	0	2	0	0	1	0	0	2	1	0	4	7
56				1	0	3	0	0	1	0	0	2	1	0	4	7
61							0	0	1	0	0	2	2	0	4	7
71							0	0	1	0	0	2	2	0	5	7
79										0	0	5	2	0	5	7
86										4	0	5	2	0	5	7
93										4	0	5	4	1	5	7

Table D.2. Cumulative number of stolonally budded polyps produced by each group across the experimental period (n=10). Column headers refer to the treatment (i.e. 2W4C = replicates were held for 2 weeks at 4 °C)

DAY	2W4C	2W7C	2W10C	4W4C	4W7C	4W10C	6W4C	6W7C	6W10C	8W4C	8W7C	8W10C	4C	7C	10C	13C
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	3	0	0	1	1	0	0	1	0	1	0	0	0	0	0
14	0	3	0	0	1	1	0	0	1	0	1	0	0	0	0	0
21	0	3	0	0	1	1	0	0	1	0	1	1	0	0	0	0
28	0	3	0	0	1	1	0	0	1	0	1	1	0	0	0	0
35	0	4	0	0	1	1	0	0	1	0	1	1	0	0	0	1
42	0	4	0	0	1	1	0	0	1	0	1	1	0	0	0	1
49				0	1	1	0	0	1	0	1	1	0	0	0	1
56				0	1	1	0	0	1	0	1	1	0	0	0	1
61							0	0	1	0	1	1	0	0	0	1
71							0	0	1	0	1	1	2	0	0	1
79										0	1	2	2	0	0	1
86										1	1	3	2	0	0	1
93										1	1	3	2	0	1	1

Table D.3. Cumulative number of podocysts produced by each group across the experimental period (n=10). Column headers refer to the treatment (i.e. 2W4C = replicates were held for 2 weeks at 4 °C)

DAY	2W4C	2W7C	2W10C	4W4C	4W7C	4W10C	6W4C	6W7C	6W10C	8W4C	8W7C	8W10C	4C	7C	10C	13C
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	5	3	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	5	3	0	0	0	0	0
21	0	1	0	1	0	0	0	0	0	5	3	0	0	0	0	0
28	0	1	0	1	0	0	0	0	0	5	3	0	0	0	0	0
35	0	1	0	1	0	0	0	0	0	5	3	0	0	0	0	0
42	0	1	0	1	0	0	0	0	0	5	3	0	0	0	0	0
49				1	0	0	0	0	0	5	3	0	0	0	0	0
56				1	0	0	0	0	0	5	3	0	0	0	0	0
61							0	0	0	5	3	0	0	0	0	0
71							0	0	0	5	3	0	0	0	0	0
79										5	3	0	0	0	0	0
86										5	3	0	0	0	0	0
93										5	3	0	0	0	0	0

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