

# **University of Southampton**

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

Ocean and Earth Science

## **Population variability in *Aurelia aurita***

by

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## Population variability in *Aurelia aurita*





UNIVERSITY OF SOUTHAMPTON

ABSTRACT

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Jellyfish blooms are increasing in some parts of the world, influencing marine ecosystem function and services for humans. Concerted research efforts have focused on understanding the causes and mechanisms of bloom events, particularly in response to environmental change. Most bloom forming species are characterised by a metagenic life cycle involving sexually reproducing medusae and asexually reproducing polyps. In comparison with the pelagic medusa little is known about polyp biology and physiology. However, the polyp is thought to be a key factor in the formation of jellyfish blooms, through the success of medusa recruitment via asexual reproduction. The study species *Aurelia aurita* is widely distributed in northern Europe and often forms bloom populations. The adaptive ability to environmental variability and change of the medusa and polyp life stage is the topic of this study.

Specifically, this PhD thesis aims to 1) understand population dynamics of the common jellyfish *A. aurita* medusa and polyp populations, 2) gain information about the physiology and reproduction of *A. aurita* polyps and 3) investigate polyp diet and metabolism under natural conditions. This study involved sampling and monitoring of natural medusa and polyp populations in southern England, and the design of ecological experiments on laboratory maintained polyps. I demonstrate that *A. aurita* follows density driven population dynamics in the Beaulieu River. Short longevity, large medusa sizes of small abundance and high mesozooplankton levels alternated with, prolonged life span, small medusae sizes of high abundance and low mesozooplankton levels. Three closely located populations of *A. aurita* in southern England varied in abundance, longevity and medusa size, thus demonstrating site-specific adaptations. Experimental studies on the effect of temperature on polyp respiration rates showed increased sensitivity to temperatures above 14°C. The respiratory response of three populations to a range of temperatures revealed evidence of acclimation to their natural thermal tolerance window. Furthermore, the asexual reproduction in response to temperature of three polyp populations differed with the lowest bud production and survival in Norwegian polyps and the greatest bud production in the southern England populations at 12 and 16°C, demonstrating polyps sensitivity to higher temperatures and adaptation to their natural environment. Furthermore, a photographic survey of *in situ* populations showed that strobilation is prolonged in Horsea Lake (Dec-Apr) with peak strobilation in January (50%). A lower number of discs were produced in nature compared to laboratory maintained polyps (4 instead of 7). Finally, I show that natural polyps are not primarily supported by zooplankton prey and switch their diet from a terrestrial and sediment derived winter diet to sediment and plankton dominated summer diet. The implications of this study were to examine adaptation in *A. aurita* populations in response to environmental parameters. Temperature was especially a critical factor on the respiratory and the reproductive rates of *A. aurita* polyps indicating a degree of sensitivity to warmer temperatures. Furthermore, reproductive rates measured on *Artemia*-fed polyps possibly overestimate medusa recruitment as polyps of the Beaulieu River were not supported by zooplankton but by (lower energy) terrestrial and sediment derived food sources. In conclusion, this thesis highlights the importance of temperature and food availability on the physiology and reproductive ability of *A. aurita* polyps, especially in the context of climate change scenarios.

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## Declaration Of Authorship

I, **Danja Persephone Höhn**,

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.

### **Population variability in *Aurelia aurita***

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
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;
5. I have acknowledged all main sources of help;
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;
7. Parts of this work have been submitted to be published as:
  - Höhn DP (2016) Will the moon jellyfish cope with future warming? *Sanctuary* p.45
  - Höhn DP, Lucas CH, Thatje S (2017) Respiratory response to temperature of three populations of *Aurelia aurita* polyps from northern Europe. *Plos One* 12(5):e0177913
  - Höhn DP, Lucas CH, Trueman C (in revision) Insights into the feeding and bionergetics of jellyfish polyps in wild and laboratory conditions: do experiments overestimate natural functional rates? *Marine Biology*

Signed:

Date:

# List of abbreviations and definitions

**AR** - asexual reproduction

**CN** - carbon and nitrogen

**DW** - dry weight

**E** - encystment

**ER** - ephyrae

**GAM** - generalized additive model

**GR** - gamete release

**ind** - individuals

**IPCC** - intergovernmental panel on climate change

**LS** - larval stage

**N/A** - not available

**pers obs** - personal observations

**RR** - respiration rates

**S** - settlement

**SD** - standard deviation

**SI** - stable isotopic

**SIAR** - stable isotope analysis in R

**ST** - strobilation

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# Chapter 1

## Introduction

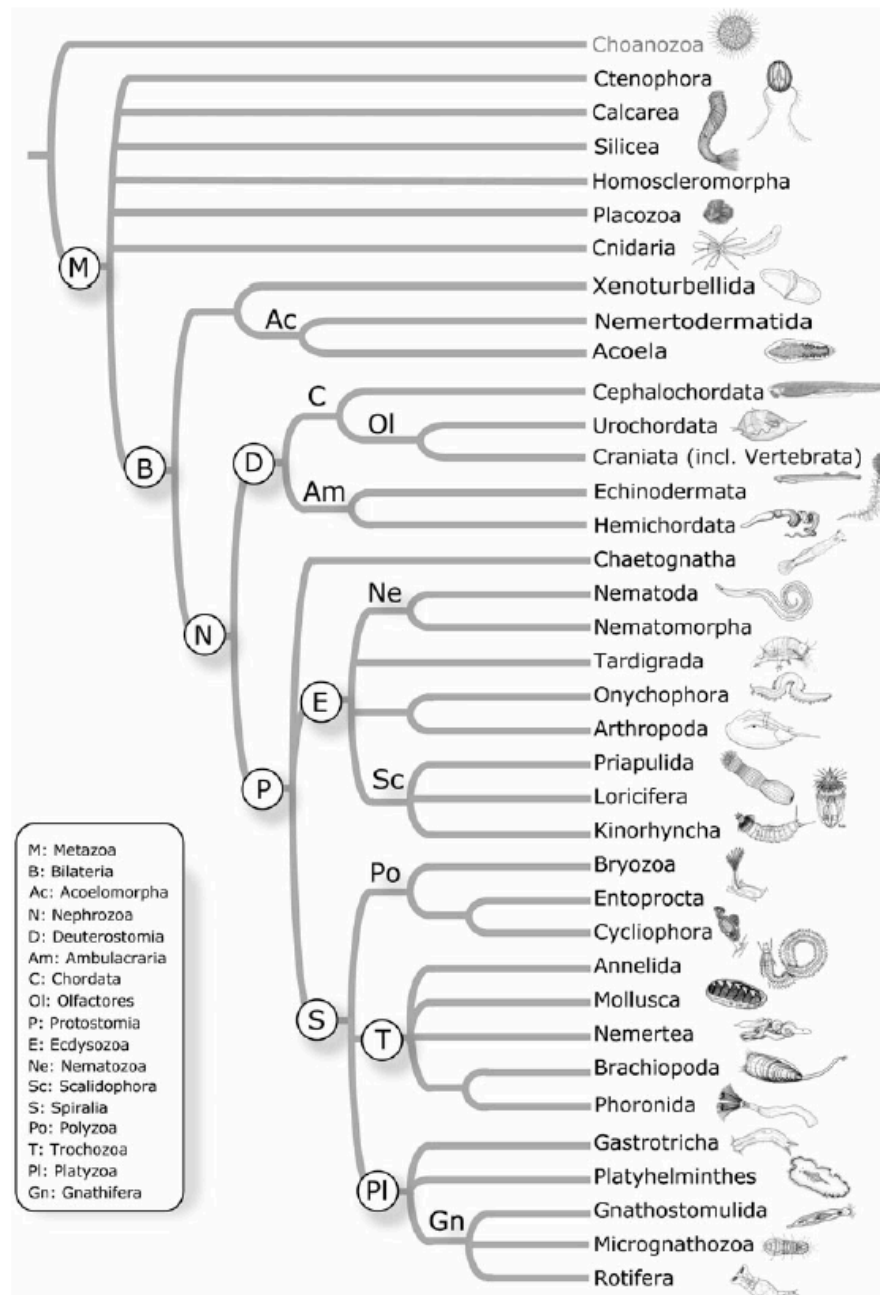
# **1. Introduction**

## **1.1 Jellyfish species**

Jellyfish belong to gelatinous zooplankton, inhabiting mainly marine but also freshwater environments and they are found in every ocean from the surface to the deep sea (Mills 1995; Lucas et al. 2014). Gelatinous zooplankton includes distinct groups of the phyla Cnidaria, Ctenophora and Chordata. These contain all clades within the annelid worms, the molluscs, the nemerteans, deep-sea fauna including holothurian echinoderms, the thaliaceans and the larvaceans in the phylum Chordata, the comb jellies in the phylum Ctenophora and the medusae and siphonophores in the phylum Cnidaria (Lucas and Dawson 2014) (Fig. 1.1).

Jellyfish species are predominantly members of Cnidaria, including 10,000 species. Members of the phylum Cnidaria are characterised by the possession of cnidae (intracellular organelles) and a body plan consisting of two epithelial body layers the epidermis and the gastrodermis, separated by mesoglea which acts like a hydrostatic skeleton and consists of water, collagen fibres and salts (a gelatinous connectivity tissue) (Arai 1997; Verde and McCloskey 1998). Many species have a bipartite life cycle alternating between a benthic polyp and a pelagic medusa. Medusae in northern temperate systems grow fast from mid- to late-spring, when the availability of food is high following the spring phytoplankton bloom (Möller 1980; Schneider 1989; Lucas and Williams 1994). Most jellyfish live for a short time (3 to 6 months) and start to degrade after gametes are released in summer, and die shortly after (Mills 1993). Food has been considered a limiting factor, and the production of gamete and the spawning process are energetically expensive processes resulting in morphological degradation and death (Marques et al. 2015; Goldstein and Riisgård 2016). Mortality is not just

caused by old age and starvation; predation, parasitism and disease also form a major contribution (Mills 1993).



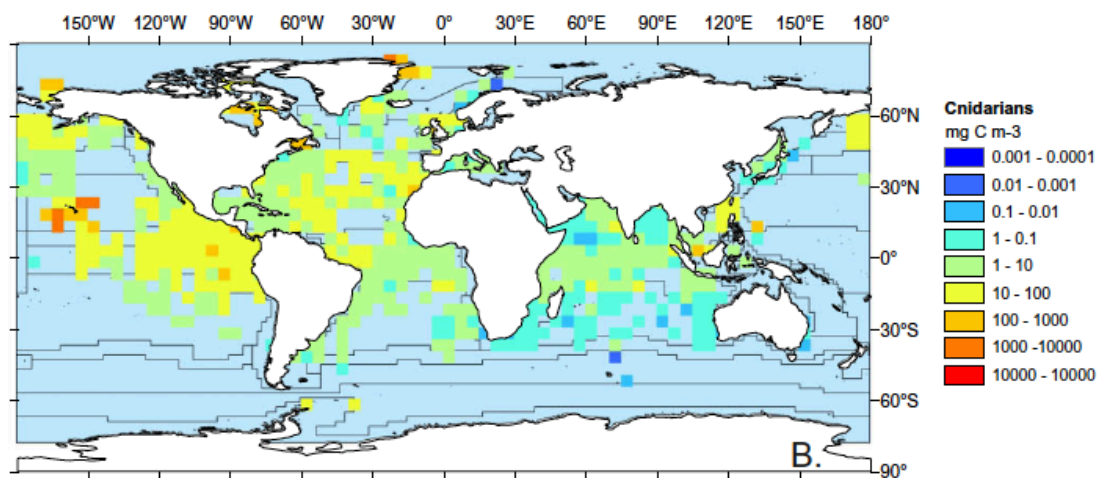
**Fig. 1.1** Gelatinous zooplankton developed about nine separate times within Metazoa. This phylogenetic tree of metazoan shows all gelatinous zooplankton phyla (Ctenophora, Cnidaria, Urchordata and Mollusca) and other phyla in which there are occasional gelatinous zooplankton taxa (Echinodermata, Annelida, Nemertea). Illustration reproduced from Edgecombe et al. (2011)

The majority of ctenophores, medusae and siphonophores are non-visual generalist feeders of vertebrate and invertebrate larvae and holoplankton including copepods and other gelatinous zooplankton (Mills 1995). Some jellyfish species select fish eggs and larvae, which is of scientific interest because medusae can prey on larvae of commercially important fish (Purcell 1997; Hansson et al. 2005). Medusae size can increase rapidly when food availability is high, and decrease during low food periods (Hamner and Jenssen 1974; Lucas 2001). Body size can be controlled by population density since large medusae have been observed to coincide with low abundance, and small medusae have been observed to coincide with high abundance (Schneider and Behrends 1998). Sea turtles (e.g. leatherbacks) mainly feed on jellyfish despite their low carbon content; consuming up to 261 jellyfish per day which makes up 73% of their body mass (Houghton et al. 2006; Heaslip et al. 2012). Jellyfish also make a major contribution to the diet of many fish species with 69 species known to have jellyfish in their gut content (Arai 1988, 2005; Pauly et al. 2009).

## **1.2 Jellyfish blooms in our oceans**

Jellyfish have the ability to grow 3.5 times faster than other animals when food availability is high and form blooms (Pitt et al. 2013). These blooms cause interactions with humans, including fisheries, aquaculture, power-generation and tourism resulting in greater scientific interest in the causes/mechanisms of these bloom events (Condon et al. 2012). Jellyfish blooms are increasing in some areas around the globe, and there are concerns that anthropogenic impacts on the environment may benefit jellyfish populations (Purcell et al. 2007). There is a scientific debate about if jellyfish blooms are increasing and there are concerns that by removing their competitors for food resources, through overfishing and ‘fishing down the marine food web’, jellyfish

abundance is likely promoted (Pauly et al. 1998; Jackson et al. 2001). In the northern Benguela Current jellyfish seem to have replaced fish and 12.2 million metric tons of jellyfish were observed compared to fish with only 3.6 million metric tons (Lynam et al. 2006). Increasing seawater temperatures, because of global warming, may increase the reproductive output and expand the distributional range of some jellyfish species (Purcell et al. 2007). However, there is evidence that jellyfish blooms are not increasing throughout the world's oceans but are part of a natural oscillation process, with some regions experiencing increases and others experiencing decreases (Lynam et al. 2011; Brotz et al. 2012; Condon et al. 2012). A new global dataset of gelatinous zooplankton records has been established for a global baseline and to understand the causes of biogeographic patterns (Lucas et al. 2014) (Fig. 1.2).



**Fig. 1.2** Map of cnidarians geometric mean biomass ( $\text{mg C m}^{-3}$ ) plotted over Longhurst biogeographic provinces. Data are given as  $5^\circ$  grid cells, and light blue indicates no observations. Illustration adapted from Lucas et al. (2014)

The formation of a bloom is often the consequence of a seasonal life cycle and usually depends on suitable environmental conditions: when food is abundant jellyfish numbers can increase rapidly and form blooms. Jellyfish blooms can also be formed by

hydrodynamic and wind-driven processes (Graham et al. 2001). There are two terminologies to describe blooms: a local increase of biomass driven by physical phenomena is called an ‘apparent bloom’, while a ‘true bloom’ is formed as a result of seasonal life cycle and is directly related to growth and reproduction (Graham et al. 2001; Lucas and Dawson 2014). Ecological and evolutionary studies are lacking and consequently the abiotic and biotic environmental parameters and the functional biology that interact to form jellyfish blooms are still not well understood. Previous studies have concentrated on local jellyfish populations because global jellyfish datasets are rare.

### **1.3 Scyphozoan jellyfish**

The Scyphozoa forms one of five classes of living Cnidaria, which have existed for over 500 million years according to fossil evidence (Arai 1997; Marques and Collins 2004; Collins and Daly 2005). Scyphozoans live exclusively in marine environments, inhabiting a wide range from the tropics to the poles and the deep-sea. Their medusa life stage dominates in most species and can be planktonic, demersal or sessile. The benthic polyp life stage attaches to a wide variety of substrates and stays exclusively attached after settlement of the planula larvae. Their life cycle is diverse and in many species the polyp is unknown or lacking, such as seen in deep-sea and open ocean species. For example, fertilized eggs in the deep-sea species *Periphylla periphylla* undergo direct development to planktonic medusa (holoplanktonic) and trachymedusae develop a free-swimming actinula larva, which assembles like a polyp without a stalk (Berrill 1952; Jarms et al. 1999). The ecology of polyps is largely unknown, compared to their pelagic medusa, on account of their small size and cryptic nature.

The Scyphozoa comprises over 200 species separated traditionally into three Orders, including the Coronatae, the Semaestomae and the Rhizostomae. The order Coronatae



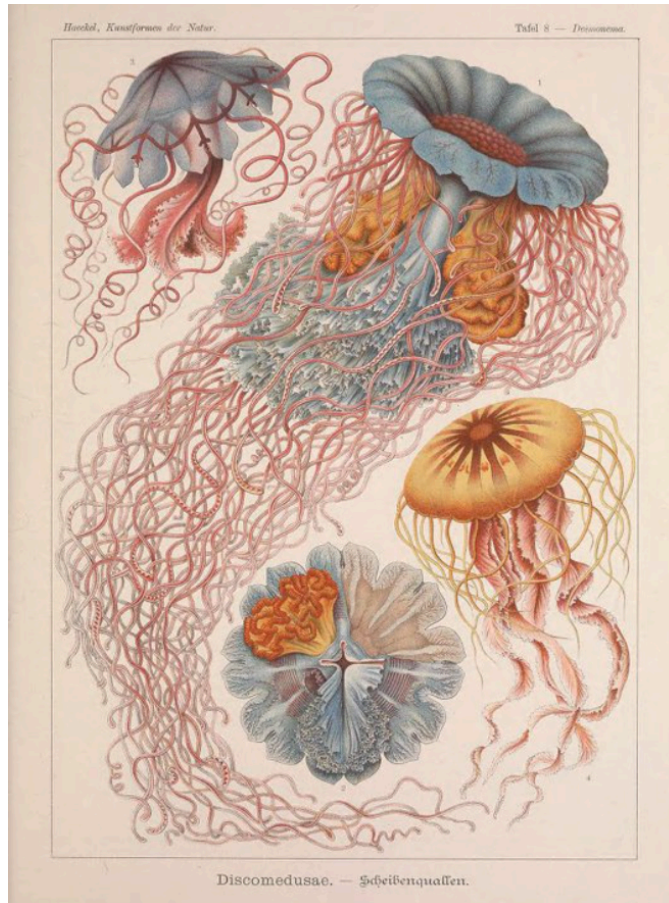
consists of seven families and includes mostly bathypelagic to mesopelagic medusa of which the life cycle is unknown (Bayha et al. 2010). Each bell has a deep furrow or coronal groove dividing the aboral surface into a central disc and a peripheral zone. This order contains *Atolla* and *Periphylla* and is considered the most primitive of the scyphozoans. The deep-sea species *P. periphylla* is red coloured, luminescent with a holoplanktonic life cycle and undergoes strong diel vertical migration (Youngbluth and Båmstedt 2001; Jarms et al. 2002).

Most of the bloom-forming species belong to the order Semaestomae and Rhizostomae (Lucas and Dawson 2014). Jellyfish of the order Rhizostomeae lack marginal tentacles and possess eight branched arms, which arise from the centre of the subumbrella (Stiasny 1920). The rhizostome *Nemopilema nomurai* (giant jellyfish) is the largest jellyfish species and occurs in Japanese and Korean Waters (Kawahara et al. 2006). The largest medusa can reach a bell diameter of 2 m, and mass occurrences in 2002 and 2003 caused serious damage to the fishing industry by clogging fishing nets (Uye 2014). *N. nomurai* is dioecious and reproduces asexually only by the formation of podocyst, and a podocyst is left behind at the previous position when moving to a new attachment (Kawahara et al. 2012).

The order Semaestomae contains most of the typical jellyfish of temperate and tropical seas and is divided into five families including Cyaneidae (*Cyanea*), Pelagiidae (*Pelagia*), Drymonematidae (*Drymonema*) and Ulmaridae (*Aurelia*) (Bayha et al. 2010) (Fig. 1.3).

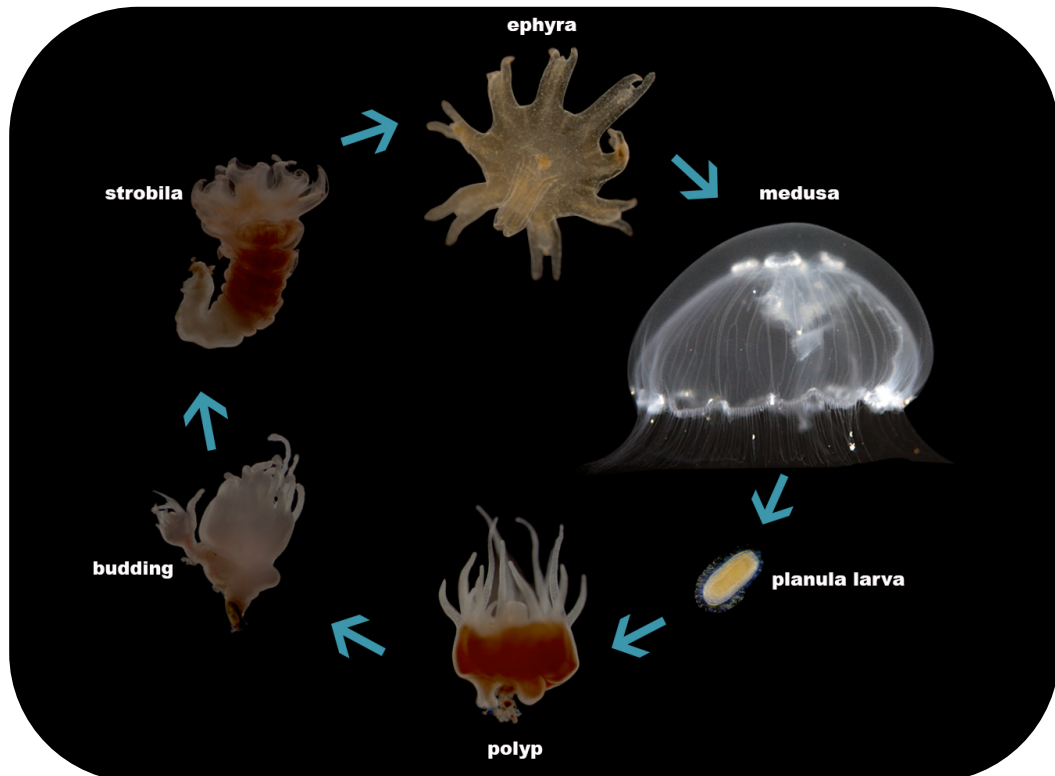
The most studied Semaestomae genus is the common bloom-forming jellyfish *Aurelia* (Bayha et al. 2010). It has been suggested that there are up to 12 cosmopolitan *Aurelia* species besides cryptic species, but the best-studied species is *Aurelia aurita* (Dawson 2003). The translucent medusa has a saucer-shaped bell, which lacks coronal groove,

pedalia and gastric septa of coronate medusa. The bell margin possesses sense organs called rophalia and is either divided into lappets or entire.



**Fig. 1.3** Scientific drawing of Semaestomae (Desmonena) produced by Eduard Giltch for Kunstformen der Natur, adapted from Haeckel's (1879) notebook

*A. aurita* medusae are well known and easily identified by their four horseshoe-shaped gonads on the aboral side of the bell. *A. aurita* has a complex, metagenic life cycle, alternating between sexual medusoid and asexual polypoid generations. The pelagic medusa broods and releases planulae larvae that settle on hard substrate and metamorphose into polyps, which will undergo asexual reproduction by budding or strobilation. During strobilation the polyp detaches pelagic ephyrae, and ephyrae will grow and mature into medusae (Fig. 1.4).



**Fig. 1.4** Metagenic life cycle of *Aurelia aurita*. The pelagic medusa releases planula larvae, which settle on the seafloor and metamorphose into polyps. Polyps undergo asexual reproduction by budding and form a strobila to release pelagic ephyrae. Ephyrae will mature and grow into medusa

Asexual reproduction has been considered to be an ‘efficient, effective and inexpensive’ process (Crow 1994), which promotes jellyfish blooms by increasing the polyp population and new recruits (ephyrae) to the medusa population. Traits of *A. aurita* and other scyphozoans including morphology and life history have favoured survival during low food availability and have increased fecundity (Lucas and Dawson 2014). Mass occurrence was preferred by such adaptations and selected for reproductive success (Dawson and Hamner 2009).

## 1.4 Variability of *Aurelia aurita* populations

### 1.4.1 *Aurelia aurita* abundance

For a long time, *Aurelia aurita* was considered a cosmopolitan species but in the early 2000s Dawson et al. (2000, 2001, 2003) identified different *Aurelia* spp. globally with

the use of DNA sequence analyses of ITS-1 (internal transcribed sequence one) and COI (mitochondrial cytochrome oxidase c subunit I). According to his work, *A. aurita* is confined to northern Europe, with other cryptic and named species identified for the Mediterranean, south-eastern Asia and North America. Habitats of *A. aurita* medusa are coastal and shelf sea environments, including coastal embayments, fjords and estuaries. Within northern Europe *A. aurita* are widespread and medusa populations have been studied in a number of locations including southern England, Southampton Water and Horsea Lake (Lucas and Williams 1994; Lucas 1996); in the Netherlands, Wadden Sea (Van der Veer and Oorthuysen 1985); in Germany, Kiel Bight (Thiel 1962; Möller 1980; Schneider and Behrends 1994); in Danish fjords (Rasmussen 1973; Olesen et al. 1994; Hansson et al. 2005; Goldstein and Riisgård 2016); in Swedish fjords (Henroth and Gröndahl 1983; Gröndahl 1988); in the North Sea (Hay et al. 1990; Lynam et al. 2004, 2005) and in the Irish Sea (Doyle et al. 2007; Bastian et al. 2014). In temperate systems, *A. aurita* follow a seasonal life cycle including the short-lived pelagic medusa and the perennial sessile polyp. During summer/autumn the sexually mature medusa releases lecithotrophic planula larvae, which will attach to hard substrates and metamorphose into polyps. Polyps start to strobilate and release ephyrae after a winter minimum of  $\sim 6^{\circ}\text{C}$ . In Fjords, ephyrae have been observed to overwinter near the bottom before they appear in the upper water layers to grow and mature into medusa during spring making the most out of the phytoplankton bloom (Rasmussen 1973; Henroth and Gröndahl 1983). During summer medusae will spawn, before they degrade and die in late summer/autumn. Although, the life cycle of European populations follows a similar pattern, interannual variability in the timing and abundance of medusae have been observed. In Southampton Water *A. aurita* maximum abundance varies from 5 to  $9\text{ m}^{-3}$ , whereas in the nearby brackish Horsea Lake abundance varied from 0.3 to  $24\text{ m}^{-3}$ .

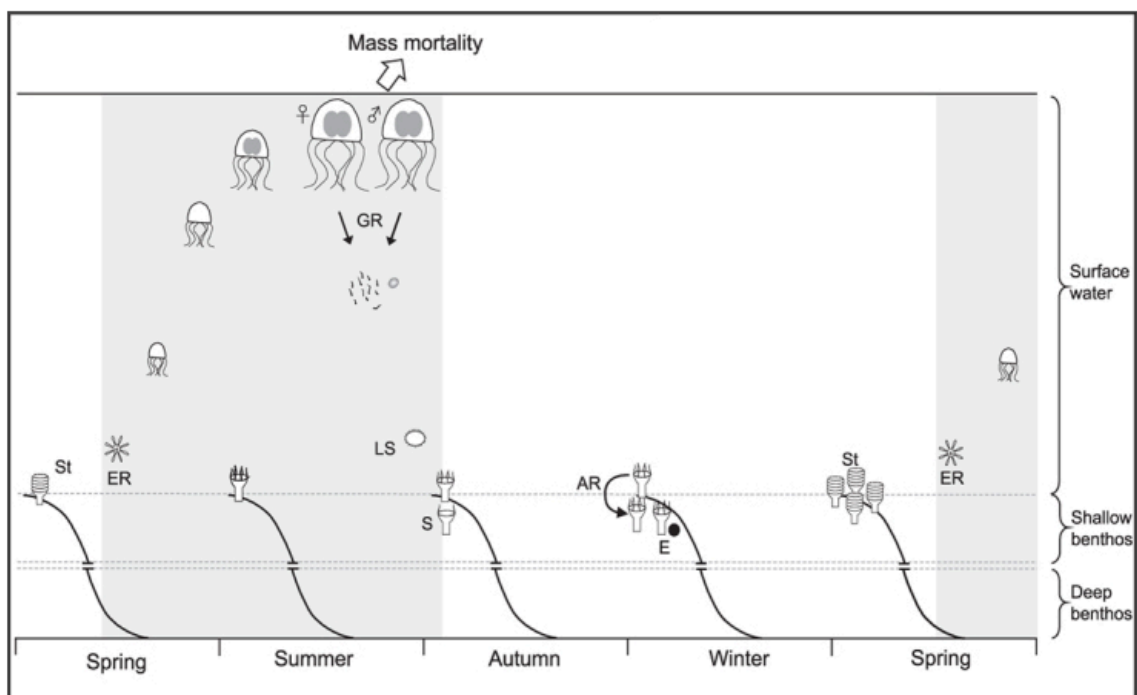
(Lucas and Williams 1994; Lucas 1996). In comparison, low medusae abundances from 0.25 to 0.5 m<sup>-3</sup> have been observed in the Wadden Sea (Van der Veer and Oorthuysen 1985). Even lower densities of 0.005 to 0.16 m<sup>-3</sup> were found in the Kiel Bight (Möller 1980; Schneider and Behrends 1994) and a density driven relationship between bell size and abundance was observed; with reduced body sizes when *A. aurita* was abundant and with large body sizes when medusae were not abundant (Schneider and Behrends 1994). In Kertinge Nor, a Danish fjord, high medusae abundances of 300 m<sup>-3</sup> *Aurelia* medusae were found during 1992 and 400 m<sup>-3</sup> during 2009 (Olesen et al. 1994; Riisgård et al. 2010). High jellyfish abundance was promoted by eutrophication caused by sewage outfall, and density might have been controlled by flush out because of water exchange processes (Riisgård et al. 2010). In Limfjorden, Denmark, a maximum medusa abundance of 61 m<sup>-3</sup> medusae was recorded in 1974, but decreased to 1.6 m<sup>-3</sup> medusae in 2003 (Hansson et al. 2005; Riisgård et al. 2012). In a small semi-enclosed bay in Norway, Vagsbopollen, *A. aurita* abundance was observed to reach a maximum of 22 m<sup>-3</sup> medusae, which is similar to the medusae abundance observed in Horsea Lake (Lucas 1996; Ishii and Båmstedt 1998). In more open water locations, for example the North Sea lower densities of 0.01 and 0.23 m<sup>-3</sup> *A. aurita* medusae were recorded and abundance was positively correlated to the North Atlantic Oscillation (Hay et al. 1990; Lynam et al. 2004, 2005). Variability in medusa abundance might depend on hydrodynamic processes and environmental parameters, including nutrient concentration, productivity and food availability.

## **1.5 Life-cycle of *Aurelia aurita* and environmental factors affecting asexual reproduction**

### **1.5.1 Polyp populations**

While the medusa of *Aurelia aurita* is tolerant to a wide range of environmental conditions of temperature, food availability, oxygen concentration, salinity and pH (Arai 1997), the sessile life stage has only been considered in a number of studies (Purcell et al. 2007; Lo et al. 2008; Prieto et al. 2010; Holst 2012). However, the polyp can exploit niche openings when other marine animals attain their physiological limits. In nature, polyp populations might increase in size greatly via asexual reproduction, but only a few studies have been conducted on natural populations (e.g. Östman 1997; Willcox et al. 2008; Purcell et al. 2009; Ishii and Katsukoshi 2010). Agassiz (1860) was the first to record the metagenic life cycle of scyphozoan jellyfish (*A. aurita* and *Cyanea capillata*), which remained in its basic structure until today but does not apply to all species (Fig. 1.5). The life cycle of scyphozoans reflects the highly seasonal environment they live in (Lucas et al. 2012): the pelagic medusa occurs when food resources are abundant and environmental conditions are favourable for growth and reproduction (Lucas 2001), whereas when resources are limited and environmental conditions degrade the sessile benthic life form persists (Boero et al. 2008). Scyphozoan medusae generally live less than a year in the wild, they grow rapidly and reach sexual maturity by summer and die after sexual reproduction (Möller 1980; Lucas 2001). However, the polyp is known to live for several years and strobilation occurs during spring, but exceptions exist with populations displaying multiple strobilation events annually (Thiel 1962; Rasmussen 1973; Möller 1980; Henroth and Gröndahl 1983; Lucas 1996). The polyp population can increase by asexual reproduction during summer through budding, during winter through encystment and in spring by

strobilation (Agassiz 1860) (Fig. 1.5). The exact timing of each event depends on changes in temperature and endogenous cues from the organism and chemical cues from the environment, since population dynamics often differ among years and populations. However, exceptions exist and the holopelagic *Pelagia noctiluca* develops ephyrae from planulae and lacks a polyp stage (Canepa et al. 2014), whereas the genus *Stephanoscyphistoma* skips the medusa stage and ephyrae directly metamorphose into planulae (Werner 1971; Jarms 1991).



**Fig. 1.5** The metagenic life cycle model from Agassiz (1860). During spring ephyrae develop into medusae. These grow through summer, reach sexual maturity, reproduce by the release of larvae and die shortly after. Planula larvae sink to the seabed, settle and metamorphose into polyps. Scyphistomae reproduce asexually through strobilation, or produce cysts. In spring, polyps develop into strobila and release ephyrae. St strobila, ER ephyrae, GR gamete release, LS larval stage, S settlement, E encystment, AR asexual reproduction. Map adapted from Ceh et al. (2015)

Polyp population size varies greatly because colonies are distributed in patches and obtaining quantitative data is difficult (Willcox et al. 2008; Ishii and Katsukoshi 2010). A high polyp density of 88 ind. cm<sup>-2</sup> was observed in Kagoshima Bay (Japan), compared to 6 - 10 ind. cm<sup>-2</sup> in the Gullmar fjord (Sweden) (Gröndahl 1988; Miyake et

al. 2002). In hypoxic bottom waters of Tokyo Bay a very low polyp population abundance of 0.0005 ind. cm<sup>-2</sup> was observed by Ishii and Katsukoshi (2010). Although seasonal data on polyp populations are extremely rare, populations can increase in spring/summer overlapping with high food availability and temperature and decrease in autumn/winter with low food availability and temperature (Willcox et al. 2008; Ishii and Katsukoshi 2010). The polyp population size in southern Tasmania was mainly regulated by food availability and density dependent factors (Willcox et al. 2008). In nature scyphozoan polyps are difficult to find, but they have been found attached on rocks, shells, polychaete tubes, ascidians, algae and bryozoan (Russell 1970; Miyake et al. 1997, 2002, 2004). However, artificial hard substrates have been named as a preferred habitat for planula larvae to settle on, and are expected to proliferate jellyfish blooms of bloom forming species including *Aurelia* spp. (Holst and Jarms 2007; Hoover and Purcell 2009; Duarte et al. 2012).

#### 1.5.2 Expansion of populations via budding

*Aurelia aurita* is found in a wide range of temperatures ranging from 0°C in Scandinavian fjords to 23°C in southern England (e.g. Horsea Lake). According to a few studies on the survivorship of polyps, temperature has the greatest effect on polyp survival such as seen in other marine invertebrate species (Willcox et al. 2007; Liu et al. 2009; Purcell et al. 2009; Klein et al. 2014). Thermal limits vary between populations and locations, depending on the annual temperature range. In Tasmania and in the Mediterranean Sea, polyp populations decrease during colder winter months (Willcox et al. 2008; Prieto et al. 2010). In Taiwan, polyps live close to their upper thermal tolerance window because a 1 - 2°C increase has been shown to cause polyp mortality (Liu et al. 2009). A big tolerance window was observed in Baltic polyps, which



survived temperatures as high as 28°C (>10°C above normal range) and increased their budding at warmer temperatures compared to their native population at 14°C (Pascual et al. 2014). However, a good understanding of thermal tolerance windows in jellyfish is still lacking, particularly in the perennial-living polyp. In summary, temperature affects all ecophysiological processes including metabolic processes (the respiration rate) of polyps, the growth rate of polyp populations and asexual reproduction (e.g. budding) (Purcell 2007; Purcell et al. 2012; Gambill and Peck 2014). While temperature and food availability have been observed to be the main conditions affecting asexual reproduction (Liu et al. 2009; Han and Uye 2010), other parameters including salinity and light (Purcell 2007; Purcell et al. 2009) and oxygen concentrations (Ishii et al. 2008) can alter the reproductive success of polyps.

In addition to strobilation (Section 1.5.3) polyps are capable of asexually reproducing in multiple ways including direct budding of new polyps, the production of stolons and hence new polyps, the production of planuloid swimming buds, longitudinal fission and the formation of cysts (Section 1.5.4) (Arai 1997). *A. aurita* populations have the potential to increase rapidly by budding new polyps because individual polyps have been observed to produce between 0.4 to 0.6 buds per day in favourable conditions (Purcell et al. 2012). Budding in *A. aurita* polyps occurs during different times in their life cycle and these seem to be linked to a variation of temperature and food availability (Liu et al. 2009; Han and Uye 2010). *Aurelia* polyps showed a higher asexual reproduction with increasing temperatures and food availability (Ishii and Watanabe 2003; Schiariti et al. 2014). This trend seems to coincide with greater growth rates of *Aurelia* polyps, which also occurs at higher temperatures (Miyake et al. 2002; Willcox et al. 2007). The availability of food affects the allocation of energy into reproduction and growth. At high food concentrations energy is allocated towards budding and the

formation of stolons, while at low food concentration energy is shifted from the production of more polyps into strobilation (Gong 2001). Starvation has been found to be the most limiting factor on asexual reproduction in *Aurelia* and other scyphozoan polyps (Schiariti et al. 2014). A similar shift in energy allocation has been reported for the medusa life stage, with an increase in somatic growth at high food availability and a switch to reproduction at low food availability (Lucas 2001). In contrast, *A. aurita* polyps from the Mediterranean Sea with an annual temperature range of 17.5 to 23°C decreased their budding rate with increasing temperatures from 15, 21 to 28°C (Prieto et al. 2010). Similarly, polyps from Taiwan propagated most buds at 20°C, the lowest temperature with a natural temperature range of 20 to 30°C (Liu et al. 2009). This indicates a high sensitivity of warm water species to environmental change because temperature changes in their natural environment are narrow. More buds are produced at high light intensities (Liu et al. 2009) but salinity has little effect on polyp and planula survival (Holst and Jarms 2010; Prieto et al. 2010). Winans and Purcell (2010) reported 100% survival of *A. labiata* polyps exposed to a combination of low pH (7.2, 7.5, 7.9) at 9 and 15°C. Polyps seem to be tolerant to low dissolved oxygen concentrations in Chesapeake Bay and Tokyo Bay (of 4.5 ml O<sub>2</sub> L<sup>-1</sup>), with the potential to outcompete other benthic marine organisms (Condon et al. 2001; Watanabe and Ishii 2001; Ishii et al. 2008).

### 1.5.3 Strobilation and medusa recruitment

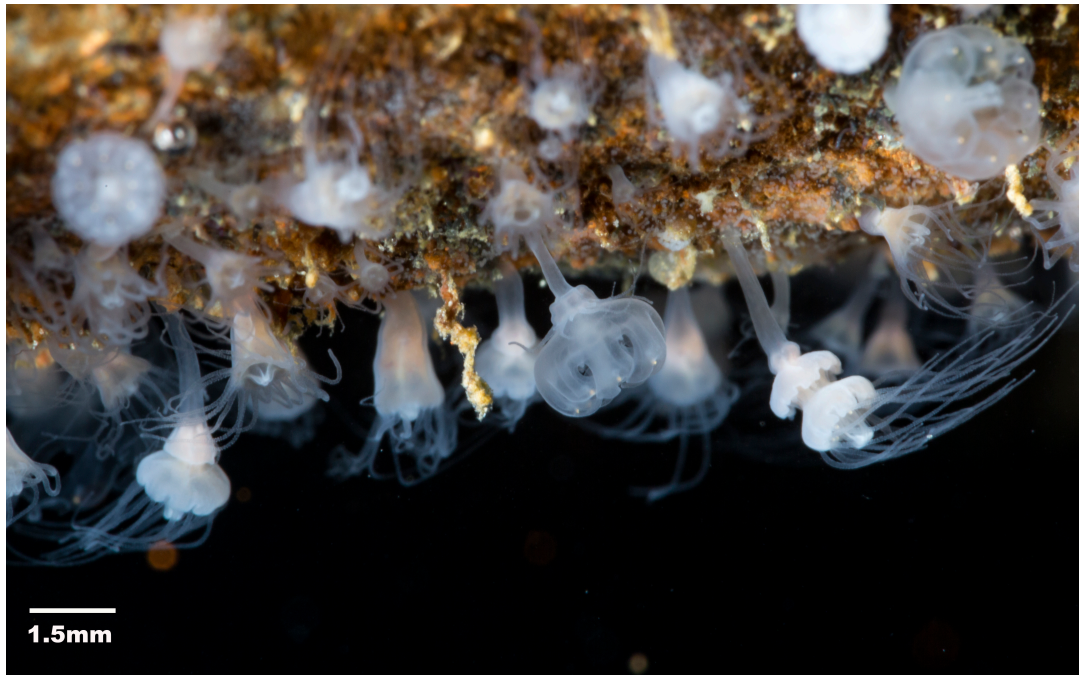
In northern European species strobilation usually occurs during late winter or early spring, months after the planula larvae have settled (Möller 1980; Henroth and Gröndahl 1985), although in wild populations, strobilation (indicated by the presence of

strobilating polyps or ephyrae in the water column) varies from location to location and from year to year (Lucas 2001).

In *Aurelia aurita* polyps a number of stimuli have been suggested to induce strobilation, including light, temperature, food, polypeptide and iodine (Spangenberg 1968). In the laboratory and in *in situ* populations strobilation has been observed during warming temperatures following a cooler period (Spangenberg 1967; Rasmussen 1973; Lucas and Williams 1994; Willcox et al. 2008).

The seasonal appearance of ephyrae throughout northern Europe in the water column indicates that an increase in temperature and food has a major effect on strobilation (Lucas 2001). Nevertheless, in the Gullmar fjord the main period of ephyrae release is during October and November, when temperatures decrease (Henroth and Gröndahl 1985). While distinct periods of strobilation have been observed in most locations, such as in Kertinge Nor (February and March), a prolonged period of strobilation occurs in the Kiel Bight (Thiel 1962; Möller 1980; Olesen et al. 1994). Similarly, a prolonged period of strobilation has been observed in the brackish water Horsea Lake in southern England (November to July) (Lucas 1996), but in the close by Southampton Water strobilation occurs once annually during winter/spring (January to March) (Lucas and Williams 1994). It seems that site-specific strobilation patterns exist in *A. aurita* populations, and this may reflect local adaptation to environmental conditions or the occurrence of cryptic species (Dawson and Martin 2001; Dawson et al. 2005). It is well reported in marine invertebrates that environmental cues play a major role in the onset and timing of their reproductive cycle and temperature is likely the main driver for strobilation. High temperatures have been shown to increase strobilation in the scyphozoan *Aurelia labiata* (Purcell et al. 2009), *A. aurita* (Liu et al. 2009; Holst 2012), *Chrysaora quinquecirrha* (Purcell et al. 1999), *Cyanea lamarckii* and *Chrysaora*

*lysoscella* (Holst 2012), and the number of ephyrae per strobila (Purcell et al. 1999; Holst 2012). There are concerns that jellyfish numbers will increase with warming since the production of ephyrae increases with temperature (Holst 2012) (Fig. 1.6).



**Fig. 1.6** *Aurelia aurita* polyps strobilating. Monodisk (with one ephyrae) and polydisk (more than one ephyrae) strobilating polyps are shown. Picture by Matt Doggett

Nevertheless, interannual differences in the onset of strobilation have been observed in well-studied *A. aurita* populations (Lucas 2001). Winter minimum temperatures that change from year to year may correlate with the timing of ephyrae release, because ephyrae production is delayed following colder winter (Rasmussen 1973). A temperature reduction of 5°C postponed strobilation in *C. quinquecirrha* by one week (Purcell et al. 1999). In contrast, Lynam et al. (2004) hypothesised that large numbers of jellyfish follow colder winters in the North Sea and that timing and intensity of strobilation is linked to the phase of the North Atlantic Oscillation (NAO). Experiments on the effects of temperature, food availability, salinity and oxygen concentration have been carried out on the number and frequency of *Aurelia* sp. strobilating. There is a

general trend of more ephyrae being produced per polyp when there is higher food availability (large, well fed polyps produce more ephyrae) (Henroth and Gröndahl 1983; Purcell et al. 1999; Willcox et al. 2008), but physiological tolerances to environmental factors such as salinity and oxygen have also been observed to affect the number of ephyrae produced. More ephyrae are produced at higher salinities (28, 36), compared to low salinities (12) in *A. aurita* polyps (Holst and Jarms 2010). Fewer ephyrae are produced at low dissolved oxygen concentrations, below 5 mg l<sup>-1</sup> (Condon et al. 2001). Intrannual variation in strobilation might also be explained by food availability (Thiel 1962), as less ephyrae were produced per polyps when starved (Han and Uye 2010). Furthermore, starvation has been named to induce strobilation (Ishii and Watanabe 2003) since zooplankton becomes scarce during winter months.

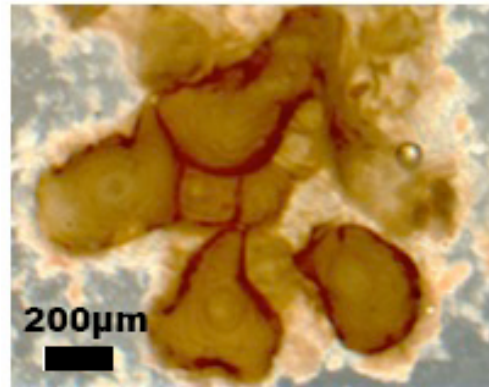
There is still a lack of knowledge about what polyps feed on in the wild and especially during winter when zooplankton stock decreases before strobilation (Thiel 1966).

Although storage products seem ecologically important for reproduction, there is not much known about the metabolism of polyps. It has been hypothesised, that polyps might not require a great amount of nutrition to build up storage products while their metabolic rate and associated costs are low during cold winter temperatures (see Kakinuma 1975). Therefore, temperature might be the most important environmental factor (in combination with food, light, salinity and DO) to induce strobilation in *Aurelia* populations, which might be adapted to local thermal tolerance windows.

#### 1.5.4 Cyst formation

Another method of asexual reproduction is the formation of chitin-covered podocysts, a dormant stage that ensures survival during extreme environmental conditions and provides protection against predation (Chapman 1968; Arai 2009; Thein et al. 2012).

Podocysts are typically irregular brown discs with a central depression on top (Holst et al. 2007) that contains stored reserves of proteins, carbohydrates and lipids (Chapman 1968; Thein et al. 2012) (Fig. 1.7).



**Fig. 1.7** Podocysts of *Aurelia aurita*

Cysts may be formed by encystment of whole polyps, by planulae as they settle (planulocysts), by pedal discs of polyps (podocysts) or by stolons in contact with the substrate (Lucas et al. 2012). Podocysts are formed during extreme environmental events for example by changes in temperature, food supply, oxygen concentration and salinity (high and low temperature), when species are reaching their physiological limits (Cargo and Schultz 1966; Gröndahl 1988). The survival of the population can be secured during periods of low food availability, by the formation of podocysts (Arai 2009). Temperature is a major environmental factor affecting metabolism and the formation of podocysts and excystment (Schiariti et al. 2014). *Aurelia aurita* s.l. polyps from the Inland Sea of Japan excysted after a 9°C temperature change from 28 to 19°C (Thein et al. 2012).

Podocysts can survive up to 3.2 years and polyps can excyst when environmental conditions improve again, to form small polyps that develop into fully active polyps, which are capable of producing more polyps (budding) and ephyrae (Thein et al. 2012). There are two major ecological roles for the production of podocysts: 1) increasing the

population size by asexual reproduction and 2) dormancy by which the population can survive for longer periods by tolerating extreme environmental condition such as temperature, hypoxia, predation pressure and low prey availability (Blanquet 1972).

### **1.6 Role of polyps in forming jellyfish blooms**

The success of the next generation depends on the successful timing of strobilation, and the recruitment into a new breeding medusa population. Polyps are perennial and have the ability to withstand changes in abiotic environmental variables, including temperature, salinity, oxygen concentration (hypoxia), light and pH. However, temperature appears to have the greatest effect on the survival of polyps, and *Aurelia aurita* show great flexibility, since they have a wide geographic distribution and survive through the cold winter months. Polyps have been observed to occur in patches of high densities under pontoons of marinas (Hoover and Purcell 2009) and on the underside of artificial hard structures (e.g. metal ladder, sunken boat) where biofouling is reduced (Höhn pers obs). In the Gullmar fjord (Sweden) polyp populations are as dense as 6 - 10 ind. cm<sup>-2</sup> (Gröndahl 1988), which likely supported mass strobilation during October and November (Henroth and Gröndahl 1985). The onset of strobilation in the Gullmar fjord seems to be triggered by a combination of decreasing temperatures and food availability. The time between the trigger to induce the transformation of the polyp into a strobila until the release of ephyrae is rapid, and transformation of the polyp can take place within a week (Purcell et al. 1999) indicating fast response and adaptability. High abundance, fast response and the ability to produce multiple ephyrae provide a mechanism for rapid expansions of medusa numbers. Multiple ephyrae can be produced either by polydisc (multiple ephyrae) strobilation, or by multiple monodisc (one ephyrae) strobilation events during a year (see Fig. 1.6). In the Gullmar fjord, monodisc

strobilation of *A. aurita* polyps in winter alternated with polydisc strobilation in spring (Henroth and Gröndahl 1985). It has been hypothesised that the amount of ephyrae produced depends on the ‘metabolic state’. During autumn polyps might be larger in size containing reserves from the summer phytoplankton bloom, which are consumed over winter resulting in smaller polyps during spring containing fewer reserves. The duration of strobilation increases with the number of ephyrae produced because ephyrae are released one by one starting at the top (Holst 2012). *A. aurita* ephyrae in the Kiel Bight (Thiel 1962; Möller 1980) and Horsea Lake (Lucas 1996) stay in the water column for seven month. However, several strobilation events might occur over a prolonged period of time, rather than a single strobilation event. The ability of *A. aurita* to form blooms, might originate from a combination of their metabolic tolerance to environmental changes in all ontogenetic life stages and a flexible life cycle that can produce a high number of ephyrae within a short period of time.

## **1.7 Metabolism**

Metabolism is difficult to measure in scyphozoan polyps due to their small size and fragile gelatinous body. *Aurelia aurita* is a well-studied scyphozoan and respiration rates have been measured (indirect way of measuring metabolism) in the pelagic life stages including medusae (Larson 1987; Frandsen and Riisgård 1997; Kinoshita et al. 1997; Uye and Shimauchi 2005; Ishii and Tanaka 2006) and ephyrae (Frandsen and Riisgård 1997; Kinoshita et al. 1997; Möller and Riisgård 2007). In contrast little information about respiration rates is available for the polyp life stage other than Mangum et al. (1972) and Gambill and Peck (2014).

Water temperature and the nutritional state of scyphozoans affect metabolic rates.

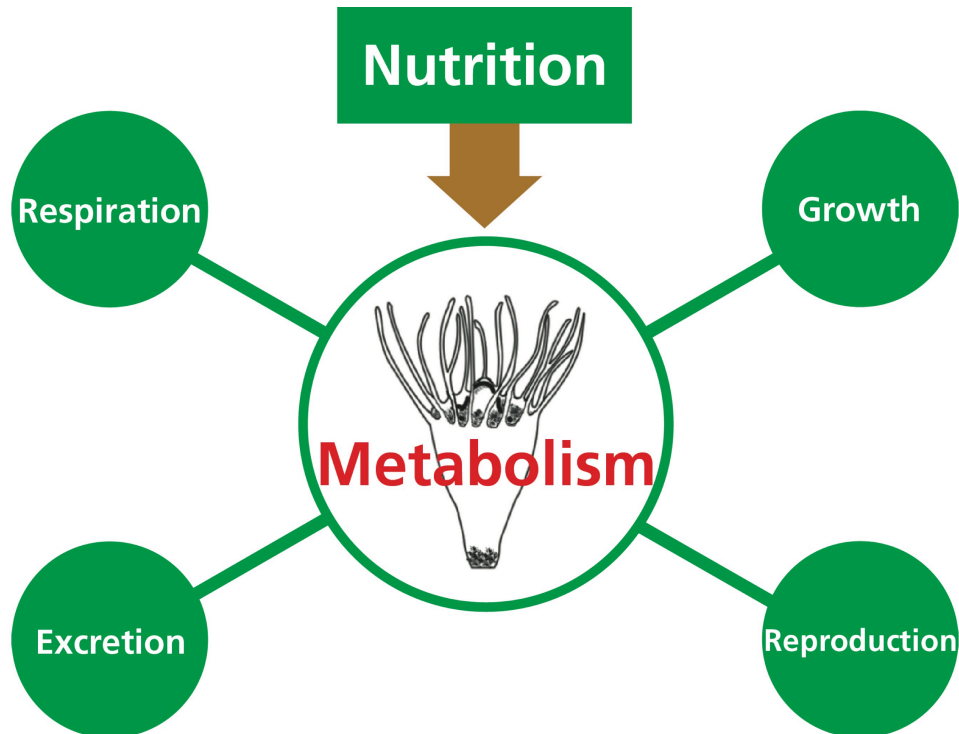
Möller and Riisgård (2007) found an exponential increase in weight-specific respiration



rates of *A. aurita* medusa and ephyrae with increasing temperatures ranging from 7 to 22°C. Furthermore, maximum respiration rates in ephyrae were observed during feeding, indicating that metabolism changes when food is abundant (Möller and Riisgård 2007). In general, respiration is believed to increase when an animal is feeding and growing to balance energy expenses that fuel biosynthesis and the assemblage of new tissue (Kiørboe et al. 1985). Metabolism includes all major ecophysiological processes such as excretion, reproduction, respiration and growth and is driven by food uptake (ingestions) (Fig. 1.8). Nevertheless, water temperature is one of the main environmental parameters that affect the metabolic rates in marine invertebrates and has been studied for some time (Thorson 1950; Childress 1971; Pearse and Lockhart 2004). Information about the metabolism of the sessile polyp life stage is scarce, most likely because of their small size and unknown locations of populations in the wild. To my knowledge, there are only two studies that have measured respiration rates in *Aurelia aurita* polyps (Mangum et al. 1972; Gambill and Peck 2014). Mangum et al. (1972) observed increased respiration rates from 12 to 32°C of *A. aurita* polyps from Chesapeake Bay (USA; temperature range 6.9 - 29°C). Whereas, Gambill and Peck (2014) found that respiration rates of *A. aurita* polyps do not increase exponentially with temperature but show a steep increase between 12 and 15°C, indicating sensitivity to warmer temperatures.

Respiration rates of other scyphozoan polyps (*Cyanea capillata* and *Chrysaora hysoscella*) did not increase with food quality (copepods) against all expectations, but growth was reduced under P-limited conditions (Lesniewski et al. 2015). In general, the size of polyps has been shown to increase with food and temperature (Ishii and Watanabe 2003; Han and Uye 2010), which is central for the development of jellyfish

blooms since the number of ephyrae produced increases with the size of polyps (Lucas et al. 2012).



**Fig. 1.8** Schematic drawing of all physiological processes that combine metabolism of polyps

However, the natural feeding ecology of polyps has not been studied *in situ* populations, but laboratory-reared polyps used for measurements of growth and asexual reproduction are regularly maintained on cultures of *Artemia* nauplii. It is important to find out the nutritional value of natural food sources to determine their actual energy metabolism in comparison with *Artemia* food.

## 1.8 Aims of the thesis

The aims of this thesis were to examine causes of population variability of the common jellyfish *Aurelia aurita* by studying the polyp as well as the medusa life stage. Natural medusa populations were studied to determine whether the study species is adapted to its surrounding environment, in terms of growth, longevity and reproduction.

Experimental work was conducted on the polyp life stage and as a measure of physiological success at different temperatures, respiration rates and reproductive rates were measured to examine acclimation processes and physiological limits of different *A. aurita* populations. *In situ* observations of polyp populations allowed the comparison of reproductive rates derived in the laboratory with wild populations.

By determining physiological tolerances in *A. aurita*, predictions can be made as to the acclimatory processes required of the polyp life stage to allow such species to form blooms in the light of rapid, ocean warming. The metabolism steers all ecophysiological processes needed to growth and reproduce and form blooms, and there is little information so far on physiological traits of polyps and how this relates to growth and asexual reproduction. Furthermore, food is another important gap of knowledge and experiments on field deployed and laboratory polyps were carried out, under summer and winter conditions, to examine their natural diet and if reproductive rates of laboratory raised polyps on *Artemia* are comparable to polyps under natural conditions. Understanding ecophysiological processes of the polyp life stage involved in reproduction is of particular relevance now because evidence is mounting that jellyfish populations are increasing in some parts of our world's oceans in response to climate warming.

### 1.8.1 Objectives

**The thesis objectives are to:**

- 1) Describe the annual life cycle of growth and development of a natural local *Aurelia aurita* population and identify population variability in growth and reproduction along well-studied closely located populations.
- 2) Measure respiration rates of *Aurelia aurita* polyps as a function of temperature exceeding their natural temperature window, to identify thermal limits and observe population specific thermal tolerances of populations from different locations.
- 3) Examine the effect of temperature on asexual reproduction of *Aurelia aurita* polyps from different locations to investigate population specific acclimation processes.
- 4) Describe the annual population dynamics of *Aurelia aurita* polyps in a natural environment and identify *in situ* abundance and strobilation activity. These values were compared with laboratory-derived observations.
- 5) Observe natural food sources for wild polyps and compare *Artemia*-fed polyps in the laboratory with wild polyps. Compare the quality between natural food sources and *Artemia* to identify natural metabolic rates of *Aurelia aurita* polyps.

# Chapter 2

**Population dynamics of *Aurelia aurita* in the Beaulieu River;  
with a comparison of three contrasting populations in the  
Solent estuarine system**

## **2. Population dynamics of *Aurelia aurita* in the Beaulieu River; with a comparison of three contrasting populations in the Solent estuarine system**

### **2.1 Abstract**

In order to understand spatial variability in the timing and extent of *Aurelia aurita* blooms, three populations have been investigated in the Solent estuarine system, southern England. In all three locations, annual *A. aurita* populations of medusae occur with recruitment of new medusae following a seasonal cycle typical for coastal European populations. During this investigation, a dense *A. aurita* population in the Beaulieu River, a tidal estuary connected to the Solent, has been studied in detail over two years 2014 - 2015 for the first time. Ephyrae started to appear in February/March after which medusae bell diameter increased linearly from March to a size of 14 cm in June 2014, after which medusae disappeared.

*A. aurita* size, abundance and seasonality varied between 2014 and 2015. Longevity was prolonged until October in 2015 and size was characterized by a small bell diameter of 5 cm. Growth (increase of bell diameter) was observed to correlate with temperature in 2014 and was inversely correlated to zooplankton abundance in 2015. The low abundance and large bell diameters in 2014, compared to the high abundance and small bell sizes in 2015 indicate that competition for food determines the inverse relationship between size and abundance of *A. aurita* in the Beaulieu River.

A comparison among Beaulieu River, Horsea Lake and Southampton Water populations within the Solent estuarine system showed intra- and interannual differences in abundance, size and seasonality. There were differences in the occurrence of ephyrae among the sites: in the Beaulieu River and Southampton Water ephyrae appeared for two months in early spring, but in Horsea Lake ephyrae appeared for seven months

following the winter. Horsea Lake *A. aurita* might have multiple overlapping cohorts as they occur all year, but in Southampton Water and in the Beaulieu River medusae only appear for 4 to 6 months. The population in Southampton Water has declined compared to 1996 as no medusae were observed between 2013 - 2017, and the reasons are unknown. This study reveals considerable variability in terms of growth, timing and reproductive behaviour in closely related *A. aurita* populations in southern England.

## 2.2 Introduction

The scyphozoan *Aurelia aurita* is a bloom-forming jellyfish found in northwest Europe. In the past, it was considered a cosmopolitan species but Dawson (2003) identified multiple cryptic species of *Aurelia* using molecular techniques, with *A. aurita* confined to northwest Europe. Medusae can be found offshore or in coastal or enclosed brackish water environments, including embayments, fjords and estuaries. These environments are often characterised by fast changing environmental conditions *A. aurita* is adapted to, including temperature, salinity, tidal cycle, and plankton abundance (Lucas 2001). *A. aurita* consumes various prey, ranging from microzooplankton to fish larvae but preys mainly on mesozooplankton (Båmstedt 1990; Arai 2001). The impact on the zooplankton standing stock by top down control can be significant, as seen in the Kiel Bight where zooplankton abundance was negatively correlated with *A. aurita* density (Schneider and Behrends 1994, 1998).

Populations of *A. aurita* in northern Europe show specific characteristics depending on their environment, indicating variability in response to environmental change. In northern Europe, populations have been documented in a number of locations: Danish fjords (Olesen et al. 1994; Goldstein and Riisgård 2016), Swedish fjords (Henroth and Grödahl 1983, 1985), Norwegian fjords (Hosia et al. 2014), the Baltic Sea (Möller

1980; Schneider and Behrends 1994), the southern North Sea (Barz and Hirche 2007) and southern England - Southampton Water and Horsea Lake (Lucas and Williams 1994; Lucas 1996).

Longevity and appearance of the pelagic phase is mainly dependent on food availability and temperature, and can vary from November to June (Southampton Water), February to October (Wadden Sea), March to December (Kertinge Nor) or be perennial (Horsea Lake and Japanese Waters) (Lucas and Williams 1994; Olesen et al. 1994; Lucas 1996; Makabe et al. 2012; Van Walraven et al. 2015). Maximum sizes of *A. aurita* medusae vary among locations and small sizes occur in Kertinge Nor (40 to 50 mm) and Gullmarfjord (55 mm), but large sizes occur in Southampton Water (300 to 400 mm) and in the North Sea (455 mm) (Grøndahl 1988; Olesen et al. 1994; Lucas and Williams 1994; Barz and Hirche 2005) (Table 2.1).

Since the early 1990s two populations of *A. aurita* have already been studied in southern England: Southampton Water and Horsea Lake, and population-specific differences have been observed (Lucas and Williams 1994; Lucas 1996) (Fig. 2.1).

Southampton Water is a 10 km long intertidal partially mixed estuary, with freshwater input from the River Test and Itchen. The estuary has great industrial activity (shipping, power stations, oil refinery) and has high productivity with a copepod secondary production of  $32.2 \text{ mg C m}^{-3} \text{ year}^{-1}$  (Hirst et al. 1999). Surface salinities vary and are about 20 at the upper part of the estuary, and between 30 and 35 in the middle to the lower part (Lucas et al. 1997). In Southampton Water the water temperature ranges from 6°C in January to 22°C in July (Lucas and Williams 1996; Muxagata et al. 2004). By contrast the man-made brackish-water 1.5 km-long Horsea Lake, in Portsmouth, has low levels of productivity. The artificial lake was designed as a torpedo-testing site, and is currently used as a Royal Navy diver training ground.



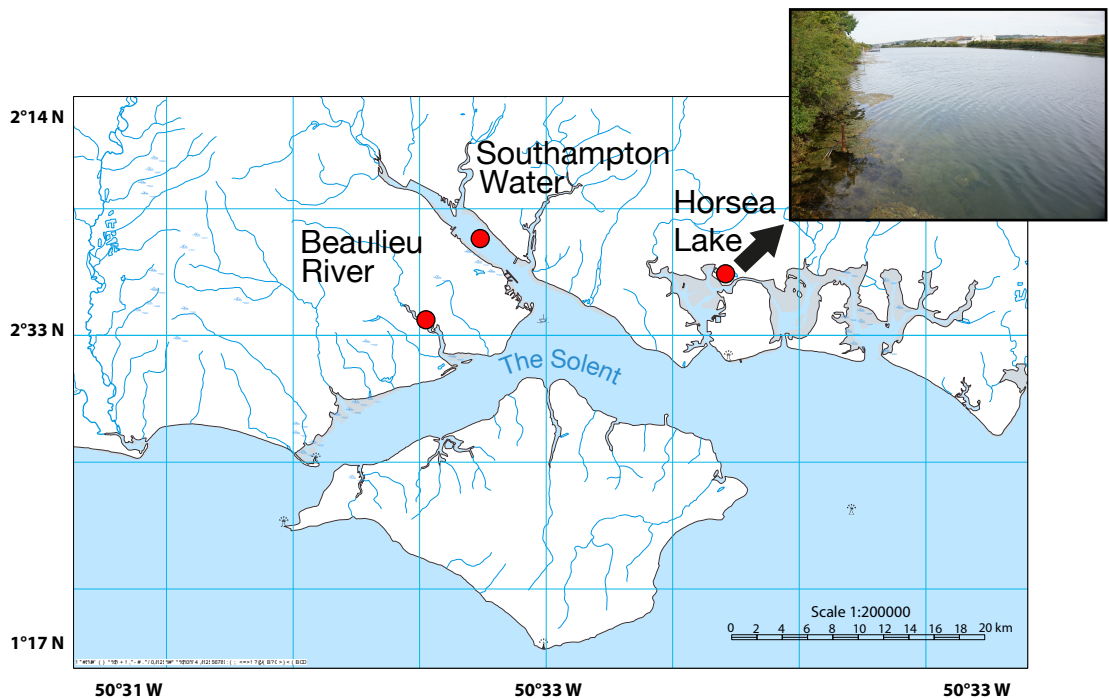
**Table 2.1** *Aurelia aurita* population dynamics in north-western Europe. NA indicates data are not available

Country	Location	Time of strobilation	Zooplankton abundance	Medusae longevity	Maximum abundance (No. m <sup>-3</sup> )	Maximum bell diameter (mm)	Size at maturity (mm)	Reference
Denmark	Kertinge Nor, Danish fjord	mid. of Feb to end of Mar (1992)	5 µg C l <sup>-1</sup>	Apr to mid. Sep	300	40-50 (Apr)	NA	Olesen et al. 1994; Riisgård et al. 1995, 2008, 2010
Denmark	Isefjord	Mar to May (2009)	Max. in spring	NA	400	30 (2009)	NA	Rasmussen 1973
Denmark	Limfjorden Nissum Bredning	NA	NA	NA	NA	NA	NA	Hansson et al. 2005
Denmark	Limfjorden Løgstør	NA	NA	NA	7	215	NA	Hansson et al. 2005
Germany	Bredning Kielfjord	NA	Max. in May and autumn; Min. early spring	Apr to Sep	61	215	NA	Möller 1980; Schneider and Behrends 1994
U.K.	Southampton Water	end of Jan to mid. of Mar	Max. in May and Oct - Nov; Min. mid-summer and winter	mid. Apr to end of Jun	8.71	150	118 (May) 64 (mid. Jun)	Lucas and Williams 1994; Lucas 2001
U.K.	Horsea Lake	Dec to end of Jun	Max. in July; Min. in winter	May to Jan	24.9	105	19 to 20	Lucas 1996
Sweden	Gullmarfjord	Nov to end of Jul	NA	Mar to Jun	24.9	55	NA	Henroth and Gröndahl 1983; Gröndahl 1988

Dutch Norway	Dutch Wadden Sea Vågsbopøl len	Oct to Mar (diapause: Jan to Mar) Feb/Mar to May	Max. in Sept-Dec; Min. in Jan - Mar May to June	Apr to mid Aug NA	14.96 22.3	NA 111.8	>156 50	Van Der Veer and Oorthuysen 1985 Ishii and Båmstedt 1998
North Sea		NA	NA	NA	22.3	NA	NA	Hay et al. 1990
North Sea	Bornholm Basin	Jul to Nov	Max. in August NA	NA	NA	455	NA	Barz and Hirche 2005
southern North Sea	55 stations	April to Aug	NA	NA	NA	NA	NA	Barz and Hirche 2007
Norway	Skagerrak	NA	only in biomass	1993 - 2005 Mar to Aug; 2006 - 2011 Mar to Nov	145 (ind. day <sup>1</sup> )	NA	NA	Hosia et al. 2014
Netherland	Dutch Wadden Sea	NA	May to Jul	NA	328 (ind. day <sup>1</sup> )	NA	NA	Van Walraven et al. 2015
Denmark	Limfjorden	Feb	in 1997 very high: 1500 µg C l <sup>-1</sup> ; in 2009 low: <50 µg C l <sup>-1</sup>	Feb to Dec	15	89-129	NA	Riisgård et al. 2012
Ireland	Irish Sea	NA	June to Aug	May to Sep	1.53 ind. 1000 m <sup>2</sup>	NA	NA	Bastian et al. 2011
Norway	Fanaafjord	NA	NA	Mar to June	100-999	NA	NA	Hosia and Båmstedt 2007
	Kertinge Nor	Mar	NA	Mar to Dec	4	250	NA	Goldstein and Riisgård 2016

The Lake is shallow with a maximum depth of 6 m, and the water temperature ranges from 5.4°C in winter to 21.8°C in summer at 6 m (present study). Saltwater only enters the lake from Portsmouth harbour when water levels drop, and salinity is brackish throughout the lake ranging from 19 to 23 due to fresh water percolating from underneath the sediment basin. Horsea Lake is a unique environment of special scientific interest due to its separation from the sea and its reduced salinity.

The Southampton Water *A. aurita* population follows a typical seasonal cycle in terms of growth and reproduction, analogous to alternative northern European populations. Ephyrae appear in late winter/early spring for about two months. Small medusae increase exponentially in bell diameter to a maximum of 300 - 400 mm in June (Lucas and Williams 1994). The peak abundance of *A. aurita* was 10 ind. m<sup>-3</sup> in June, and declined rapidly afterwards until disappearance in July (Lucas and Williams 1994).



**Fig. 2.1** Location of Southampton Water, Horsea Lake and Beaulieu River

In contrast, Horsea Lake ephyrae started to appear in December and stayed in the water column for seven months, indicating an extended time of strobilation (Lucas 1996). In Horsea Lake, medusae are known to persist throughout the year, with small bell diameters of <50 mm and peak abundance of 25 ind. m<sup>-3</sup> (Lucas 1996).

The Beaulieu River is an area of environmental protection, and flows for 19 km south westwards through the New Forest into the Solent. The river drains 80 km<sup>2</sup> of heathland and bog resulting in organic-rich water (Turner et al. 1998). The estuary is surrounded by saltmarshes and mudflats and consists of a tidal height of 4.4 m (Chen et al. 2011). A third population of aggregated *A. aurita* is found in the Beaulieu River. In calm weather conditions, there is low tidal current velocity (<0.1 m s<sup>-1</sup>) and low wave action (Chen et al. 2011). Environmental parameters, including temperature and salinity, are variable and characteristic of an estuary system. The aggregation of *A. aurita* has been observed to move up and down the mid-part of the estuary following the tide (Phillips 2012). After repeated surveys of the river from the mouth to the middle- to upper section, it has been proposed that this jellyfish population is confined to the Beaulieu River with little or no immigration from the Solent, although this has yet to be rigorously tested.

All three locations inhabited by *A. aurita*, Southampton Water, Horsea Lake and the Beaulieu River, are closely situated on a geographic scale and are part of the Solent estuarine system, a 20-mile long strait that separates the Isle of Wight from the mainland of Hampshire. For the first time, records of the *A. aurita* population and environmental components from the Beaulieu River from 2014 - 2015 are presented and compared with historical data from closely related populations from Southampton Water and Horsea Lake. *A. aurita* in Southampton Water and Horsea Lake have already

been described by Lucas and Williams (1994) and Lucas (1996), but they have not been compared with the Beaulieu River population before.

It is hypothesised that there are no inter- and intraannual differences between *A. aurita* populations. All three *A. aurita* populations are compared and the abiotic environmental differences are assessed and discussed. Furthermore, it is hypothesised that populations will not display differences in population dynamics, in relation to changing environmental variables.

## **2.3 Materials and methods**

### **Seasonal occurrence and environmental parameters in the Beaulieu River**

#### **2.3.1 Abiotic sampling and measurements**

Temperature (°C), salinity and dissolved oxygen concentration (ml/L) measurements were made at Buckler's Hard Marina, Beaulieu River (50° 80.0455 N / 1° 42.2812 W) at half-meter intervals from the surface to 4 - 5 m depth, using a hand-held YSI probe (600QS). The depth of the estuary varied depending on the tidal stage. A temperature logger (nke instrumentation, S2T600) was placed sub-surface at the underside of the pontoon, logging temperature data every 5 minutes from May 2014 to January 2016. Three times, 50 ml surface estuarine water was filtered through a 25 mm 'Whatman' GF/F filter (0.7 µm pore size) at each sampling event. Filters were covered instantly in tin foil and transported on ice to the National Oceanography Centre and placed into the -80°C freezer, where they remained until analysis. Chlorophyll was extracted in 10 ml of 90% acetone, and the fluorescence was measured using an Aminco 10–20 fluorometer with correction of phaeopigments (see Parsons et al. 1984).

### 2.3.2 Biotic sampling and measurements

The seasonal occurrence of *Aurelia aurita* was investigated by bi-weekly sampling activity at Buckler's Hard Marina, during 2014 and 2015. Bucklers' Hard Marina is located approximately 1/3 way up from the river mouth (Fig. 2.1). Zooplankton samples were collected 2 h before high water. A 210  $\mu\text{m}$  mesh size, 50 cm diameter plankton net fitted with a mechanical flow meter (Hydro-Bios) was used, to tow the net at subsurface depth along the pontoon for about 3 to 10 minutes. The tow was repeated three times. The length of the tow was adjusted in order to prevent clogging of the net once medusae were abundant. The mesozooplankton was preserved in 500 ml plastic bottles with 4% seawater buffered formalin. A 10% subsample of the zooplankton sample was identified using a low light stereomicroscope and a Bogorov chamber.

*A. aurita* ephyrae abundance was assessed in each sample by filtering the content of the bottle through a sieve. The number of medusa in each haul was counted, and particular note was taken of their reproductive state. Bell diameters were measured on preserved specimens in the laboratory. Biomass of *A. aurita* was calculated by utilising the length: weight regression given by Lucas and Williams (1994) to convert bell diameter to weight. Rainfall data were obtained from [www.southamptonweather.co.uk](http://www.southamptonweather.co.uk).

### 2.3.3 Statistical analysis

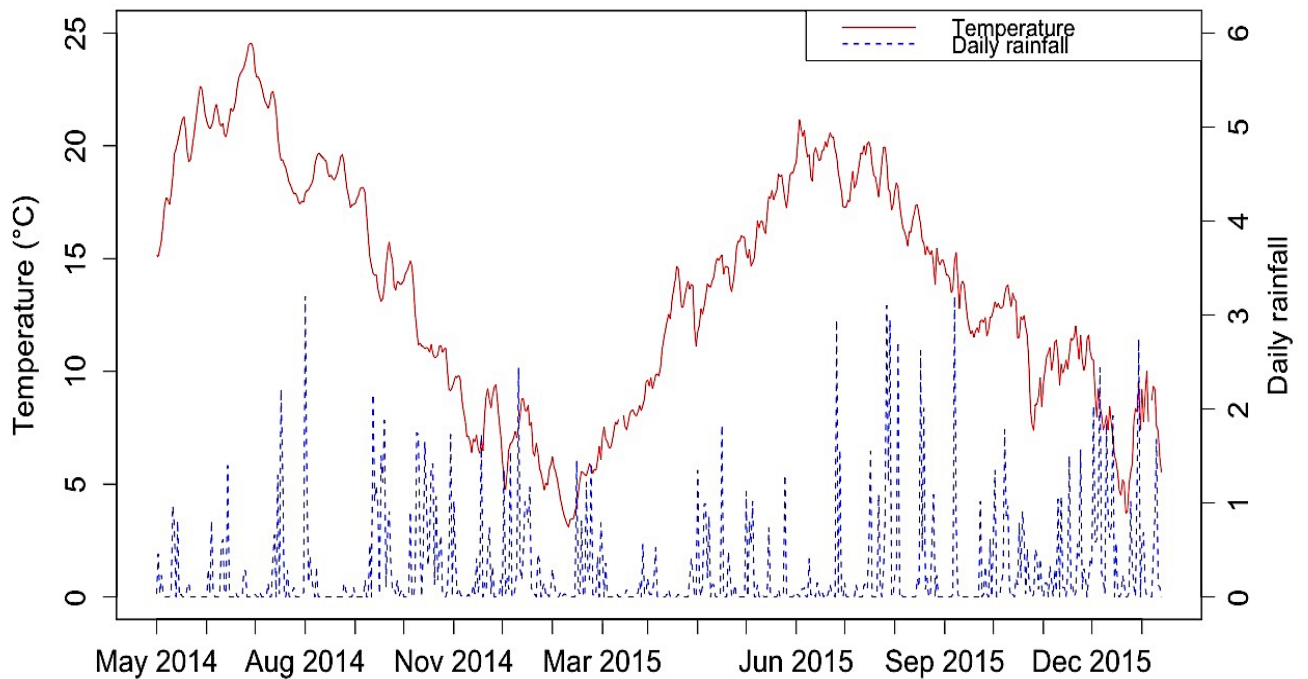
Pearson r correlation was carried out between sizes, temperature, zooplankton abundance and medusae abundance. Pairwise t-tests were performed in order to observe differences between the years. Linear regressions for sizes were performed. One-way ANOVA was performed to test for differences between the sizes, medusae abundance and zooplankton abundance of each population.

## 2.4 Results (Beaulieu River – Buckler’s Hard Marina)

### 2.4.1 Environmental parameters

Daily mean surface water temperatures in 2014 ranged from 4.75°C in December to 24.56°C in July (Fig. 2.2). In 2015, the water temperature was significant lower, ranging from 3.11°C in February to 21.17°C in July (t-test:  $t=8.58$ ,  $df=579$ ,  $p<0.0001$ ) (Fig. 2.2). Daily rainfall ranged from 0 to 3.2 between May 2014 and December 2015.

The pH in the Beaulieu River varies from 7.8 (May 2014) to 8.83 (April 2015), with an average of 8.35.

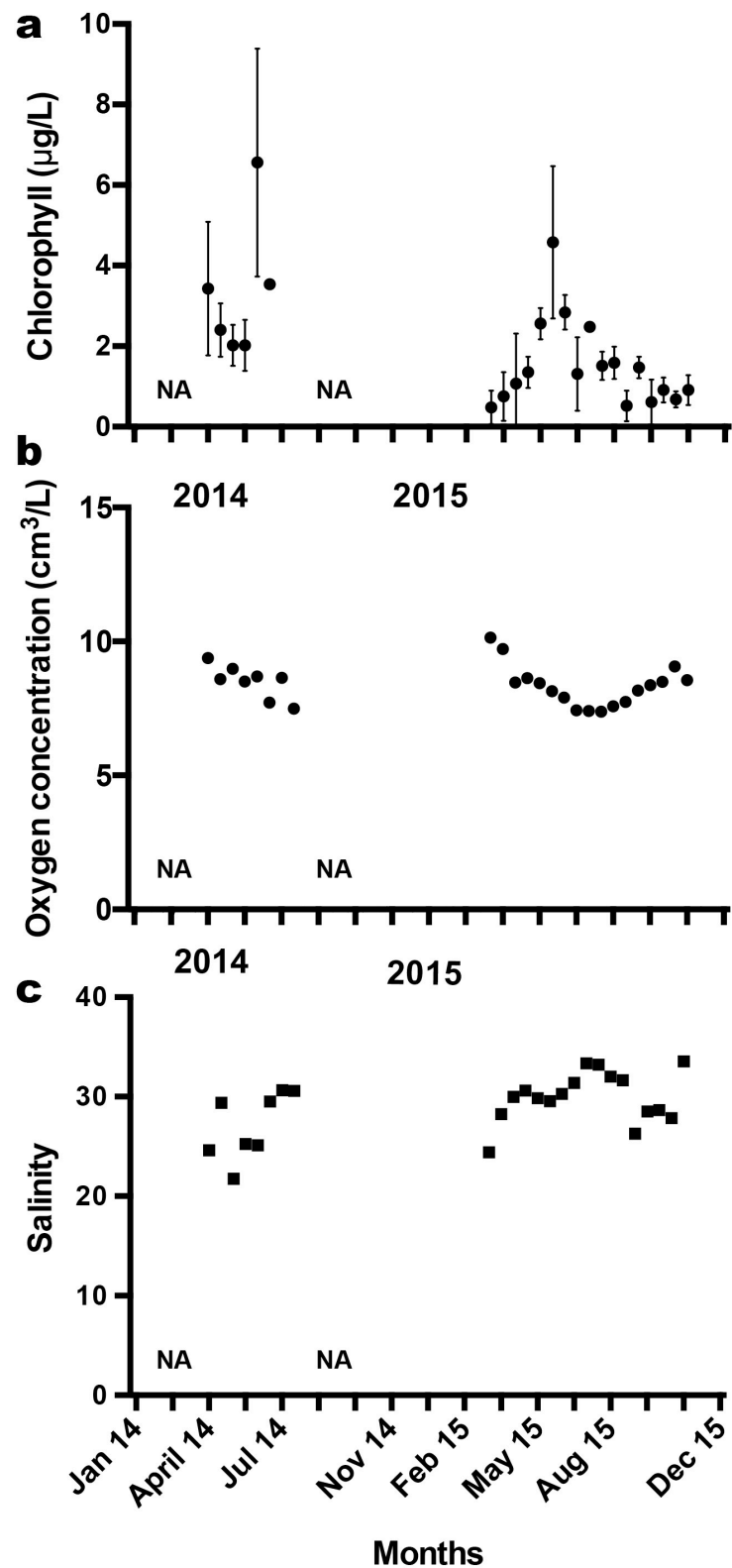


**Fig. 2.2** Daily mean surface water temperature (°C) and daily rainfall at Buckler’s Hard in the Beaulieu River, from 28th of May 2014 to 10th of February 2016. Temperature data were logged continuously, with discrete measurements taken every 5 minutes

In 2014, the minimum chlorophyll concentration was 2.02  $\mu\text{g/L}$  at the end of May and was at a maximum of 6.56  $\mu\text{g/L}$  at the end of June. The average chlorophyll concentration from April to July was 3.33  $\mu\text{g/L}$  (Fig. 2.3 a). During 2015, chlorophyll concentrations were at a minimum of 0.48  $\mu\text{g/L}$  at the beginning of March, then they increased to a maximum of 4.59  $\mu\text{g/L}$  at the end of May before decreasing again. The average chlorophyll concentration over the sampling period from March to October was 1.51  $\mu\text{g/L}$  (Fig. 2.3 a).

The average oxygen concentration in the Beaulieu River from April to May 2014 was  $8.1 \pm 0.64 \text{ cm}^3/\text{L}$  with a minimum of 7.2  $\text{cm}^3/\text{L}$  at the end of June and a maximum of 9.4  $\text{cm}^3/\text{L}$  in April (Fig. 2.3 b). In 2015, the average oxygen concentration was  $8.3 \pm 0.74 \text{ cm}^3/\text{L}$  from March to October with a minimum of 7.26  $\text{cm}^3/\text{L}$  oxygen concentration at the end of July and a maximum of 10.2  $\text{cm}^3/\text{L}$  in March. In general, salinity varied greatly in the Beaulieu River on a spatial and temporal scale with low salinities in spring but high salinities during summer. Salinity at Buckler's Hard Marina ranged from 21.76 in May to 30.64 at the end of June with an average of 27.15, in 2014. During 2015, salinity fluctuated from 24.43 in March to a maximum of 33.54 in October, with an average of 29.97 (Fig. 2.3 c).



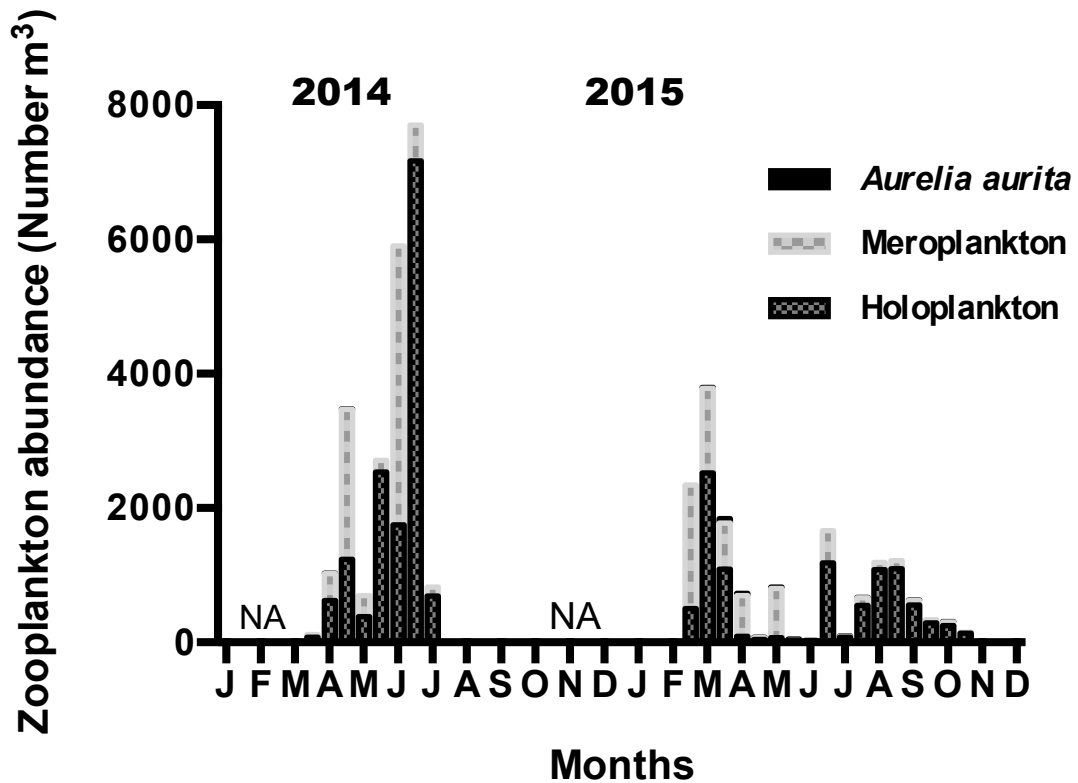


**Fig. 2.3** Seasonal environmental data from the Beaulieu River in 2014 and 2015. **a**: mean  $\pm$  sd chlorophyll concentrations; **b**: depth-averaged oxygen concentrations  $\pm$  sd; **c**: depth-averaged salinities  $\pm$  sd. NA indicates that data are not available for this period

#### 2.4.2 Zooplankton abundance and composition

The mesozooplankton standing stock in the Beaulieu River was composed of: copepods, barnacle larvae, polychaete larvae, mollusc eggs and larvae, gelatinous zooplankton including *Aurelia aurita*, ctenophores and hydrozoans, decapod larvae, fish eggs and larvae, nematodes, appendicularians, amphipods and chaetognaths.

In 2014 total zooplankton abundance increased from 119.42 ind. m<sup>-3</sup> in April to 7704.34 ind. m<sup>-3</sup> at the end of June before it decreased in July to 3061.62 ind. m<sup>-3</sup> (Fig. 2.4). In 2015, there were multiple peaks in total zooplankton abundance, with little evidence of a unimodal seasonal pattern. Minimum zooplankton abundance of 32.40 ind. m<sup>-3</sup> occurred at the end of June, with a maximum total zooplankton abundance of 3797.11 ind. m<sup>-3</sup> during March. The average total zooplankton abundance was 614.40 ind. m<sup>-3</sup>, in 2015. Two zooplankton peaks (spring and autumn) are visible in 2015, with the first and highest peak in March - April and the second in August - September (Fig. 2.4). There was a significant difference in zooplankton abundance between 2014 and 2015 (unpaired t-test:  $t=2.52$ ,  $df=23$ ,  $p=0.02$ ).



**Fig. 2.4** Total zooplankton abundance and composition of holoplankton (ctenophores, copepods, hydrozoans, fish larvae, nematodes, fish eggs, appendicularians, amphipods, chaetognaths), meroplankton (barnacles, polychaetes, decapodes, mollusc larvae, mollusc eggs) and *Aurelia aurita* during 2014 and 2015. NA indicates no sampling activity. Copepods and barnacle larvae dominated the zooplankton community

#### 2.4.3 Population dynamics and reproductive cycle of *Aurelia aurita*

The Beaulieu River consists of a locally dense population of *Aurelia aurita*, which passes upstream at Buckler's Hard Marina approximately two hours before high tide. *A. aurita* abundance data represent an aggregation of medusae formed by river currents and the tidal cycle of the adjoining Solent estuarine system.

In 2014 the average bell diameter of *A. aurita* medusae increased linearly from 0.32 cm in April to 14.4 cm in June ( $Y=0.3237 \cdot X+1.438$ ) (Fig. 2.5). In April, the population was characterised by ephyrae and juvenile medusae. Bell diameters were only measured in medusae, as ephyrae had a very small size of <2 mm. During 2014, the reproductive phase was short: mature and spent medusae were first observed at the end of May and persisted until the end of June (Fig. 2.5). After June medusae had disappeared from the

water column indicating the end of medusae's life. *A. aurita* abundance was low in 2014, ranging from 7.55 ind. m<sup>-3</sup> at the end of April to 0.26 ind. m<sup>-3</sup> in the middle of May (Fig. 2.5). Medusa size was significantly correlated with temperature (Pearson r:  $r=0.90$ ,  $r^2=0.81$ ,  $p=0.01$ ), but size was not correlated with zooplankton abundance (Pearson r:  $r=0.73$ ,  $r^2=0.54$ ,  $p=0.09$ ), or jellyfish abundance (Pearson r:  $r=0.38$ ,  $r^2=0.14$ ,  $p=0.46$ ).

In 2015, medusa average bell diameters were significantly smaller compared to 2014 (unpaired t-test:  $t=2.15$ ,  $df=18$ ,  $p=0.05$ ). *A. aurita* sizes increased linearly from April to October ( $Y=0.1780 \cdot X+0.7705$ ) with bell diameters ranging from 0.65 cm in April to 4.58 cm at the end of September (Fig. 2.5).

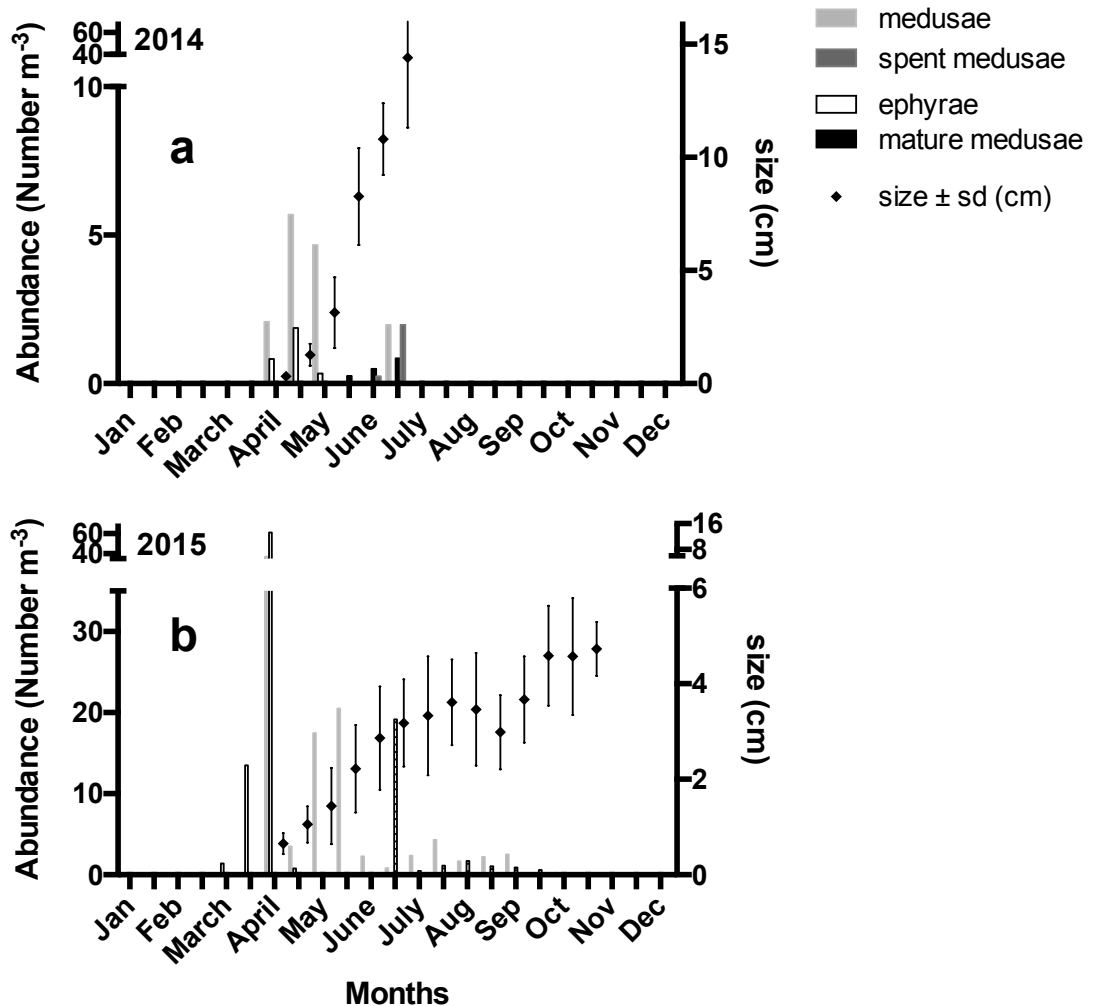
Ephyrae were present in the water column from the beginning of March until April 2015, indicating a shorter strobilation period. Ephyrae grow and mature in the spring, until they reach their reproductive phase in June. Their reproductive phase was extended during 2015, which started with the occurrence of mature medusae in the water column at the end of June and continued until the end of October.

Total medusae abundance in 2015 was higher compared to the previous year and ranged from 0.29 ind. m<sup>-3</sup> in October to 61.18 ind. m<sup>-3</sup> in April. However, there was no significant difference in medusae abundance between 2014 and 2015 (unpaired t-test:  $t=1.26$ ,  $df=20$ ,  $p=0.22$ ). Medusae sizes were inversely correlated with jellyfish abundance and sizes increased while the jellyfish abundance decreased (Pearson r:  $r=0.76$ ,  $r^2=0.58$ ,  $p=0.001$ ). Sizes were not significantly correlated to temperature (Pearson r:  $r=0.12$ ,  $r^2=0.02$ ,  $p=0.69$ ) or zooplankton abundance (Pearson r:  $r=-0.3$ ,  $r^2=0.09$ ,  $p=0.29$ ).

Ephyrae were only present in the water column at the beginning of April, in 2015.

Medusae abundance stayed high from April to July, before it declined rapidly to very

low levels at the end of July ( $1.31 \text{ ind. m}^{-3}$ ). Medusae abundance was low from July onwards until disappearance in October ( $0.39 \text{ ind. m}^{-3}$ ) 2015 (Fig. 2.5).

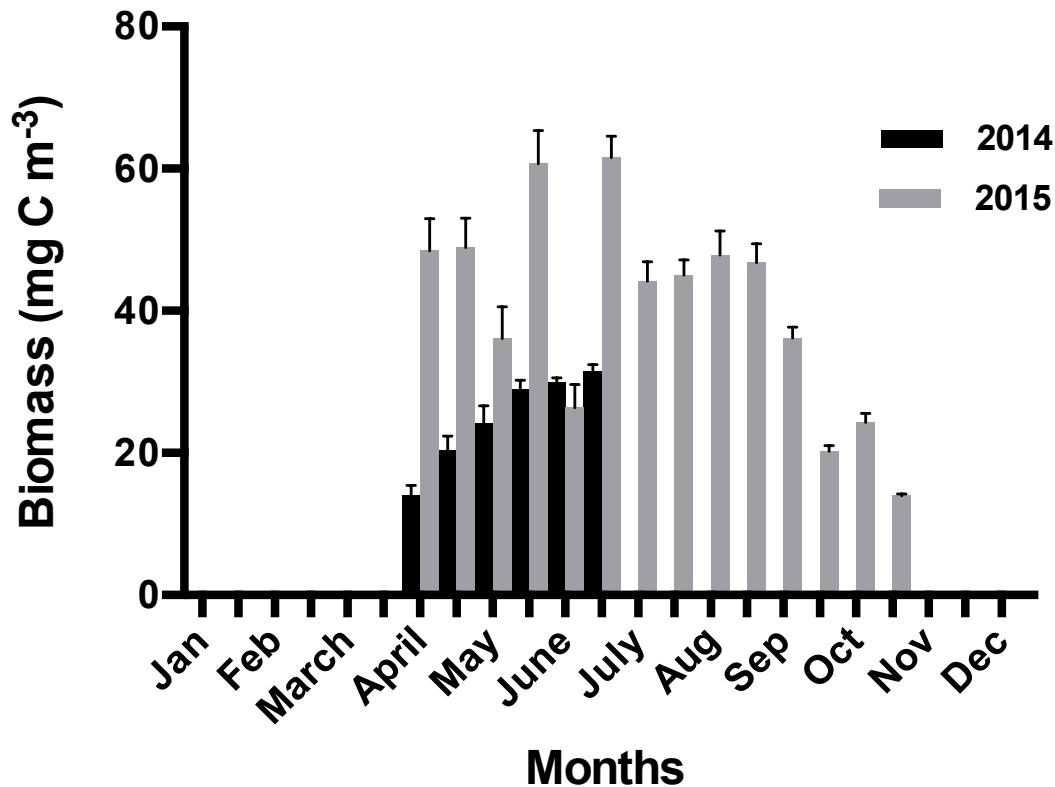


**Fig. 2.5** *Aurelia aurita* medusa abundance and mean sizes  $\pm$  sd in **a:** 2014 and **b:** 2015. The reproductive state of medusae is indicated by the colour of the bar (see legend)

#### 2.4.4 Biomass of *Aurelia aurita*

*Aurelia aurita* biomass differed significantly between 2014 and 2015 (unpaired t-test:  $t=2.44$ ,  $df=8$ ,  $p=0.03$ ). Average biomass increased from  $14.04 \pm 1.41 \text{ mg C m}^{-3}$  in April to  $31.45 \pm 0.96 \text{ mg C m}^{-3}$  in June 2014 (Fig. 2.6). During 2015, biomass was higher with values ranging from a maximum of  $61.56 \pm 2.99 \text{ mg C m}^{-3}$  in June to a minimum

of  $13.97 \pm 0.28 \text{ mg C m}^{-3}$  in October. The highest *A. aurita* biomass was between April and August, with values declining from August until October.



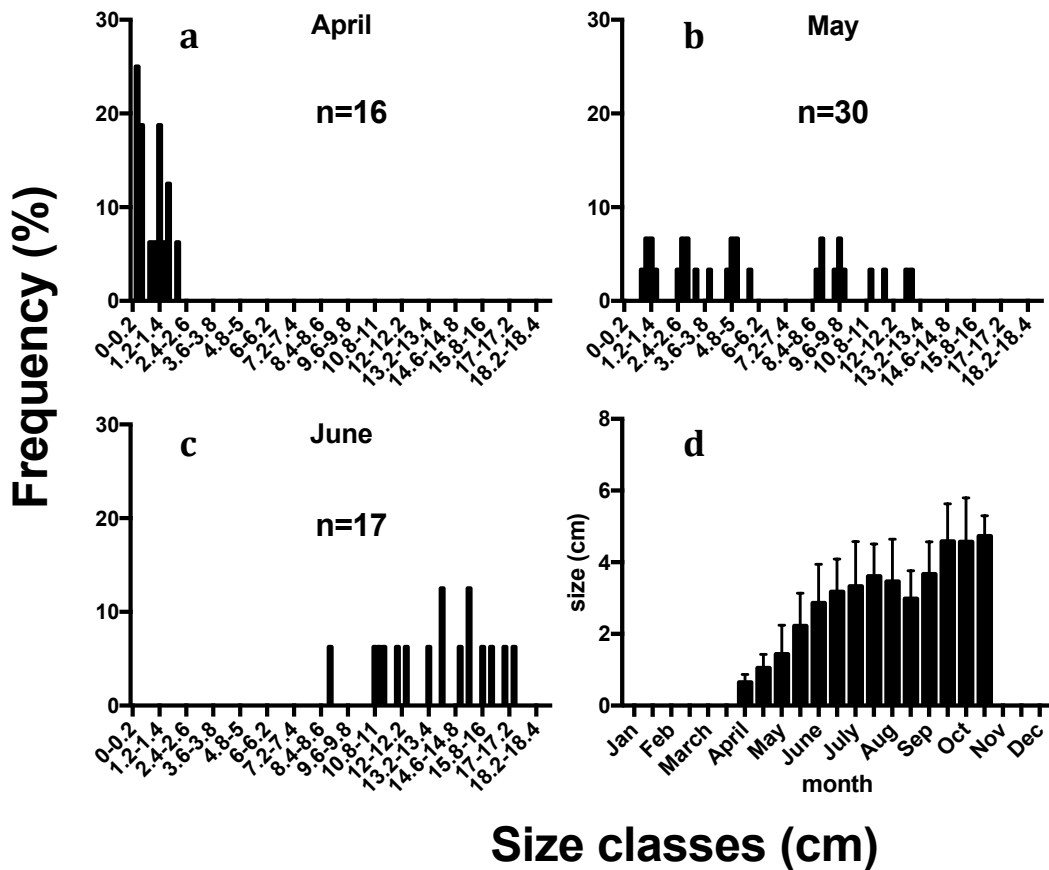
**Fig. 2.6** *Aurelia aurita* biomass. Mean biomass  $\pm$  sd ( $\text{mg C m}^{-3}$ ) of Beaulieu River medusae in 2014 (grey bars) and 2015 (black bars)

#### 2.4.5 Size-frequency histograms

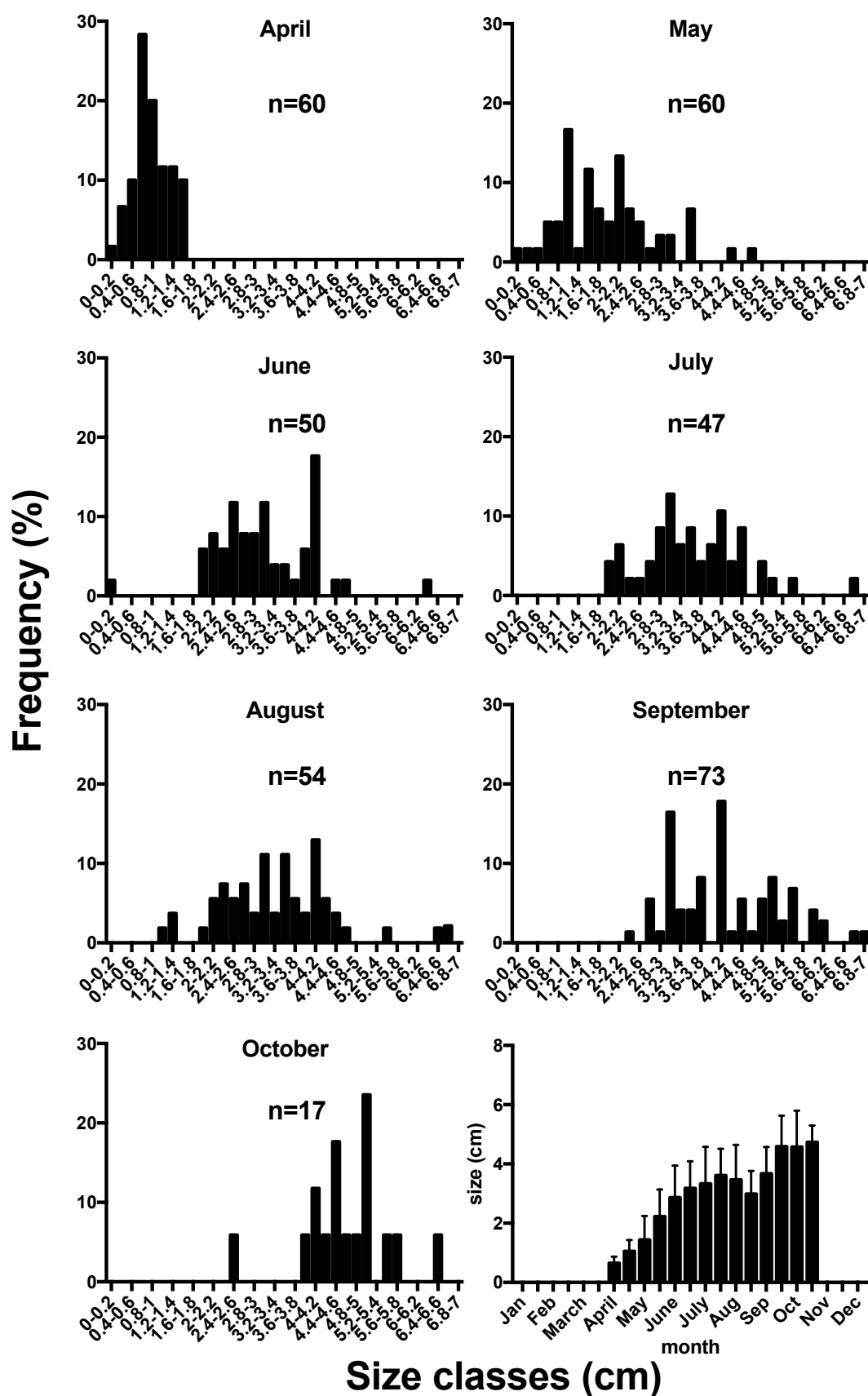
Bell diameters of medusae increased from spring onwards until they disappeared in summer (2014) and autumn (2015) (Fig. 2.7 and 2.8). In April 2014, *Aurelia aurita* had small bell diameters ranging from 0.2 to 2.2 cm (Fig. 2.7). In May, bell diameters ranged from 1.2 to 13.2 cm. In June, largest bell diameter sizes were observed varying from 9.2 to 17.6 cm (Fig. 2.7). Sizes were significantly different among the three months (One-way ANOVA:  $F_{2,60}=77.15$ ,  $p<0.0001$ ).

In 2015, overall bell sizes were small with the greatest changes from April to October (Fig. 2.8). In April, sizes were at their minimum and size classes showed a unimodal

distribution with values ranging from 0.2 to 1.6 cm. This pattern changes in the following months when bell sizes increased, but the small sizes stayed apparent resulting in a wide size range. The largest bell sizes were displayed in September with values ranging from 2.4 to 7 cm. However, the greatest proportion of large bell diameters occurred during October (Fig. 2.8). There was a significant difference between monthly sizes (One-way ANOVA:  $F_{6,354}=99.05$ ,  $p<0.0001$ ) (Fig. 2.8).



**Fig. 2.7** *Aurelia aurita* size-frequency histograms from April to June 2014 (a - c) and mean size  $\pm$  sd (cm) versus month (d). Size classes scale from 0 to 18.4 cm and sample numbers (n) are shown in each graph. Sizes were significant different between April, May and June (One-way ANOVA:  $F_{2,60}=77.15$ ,  $p<0.0001$ )

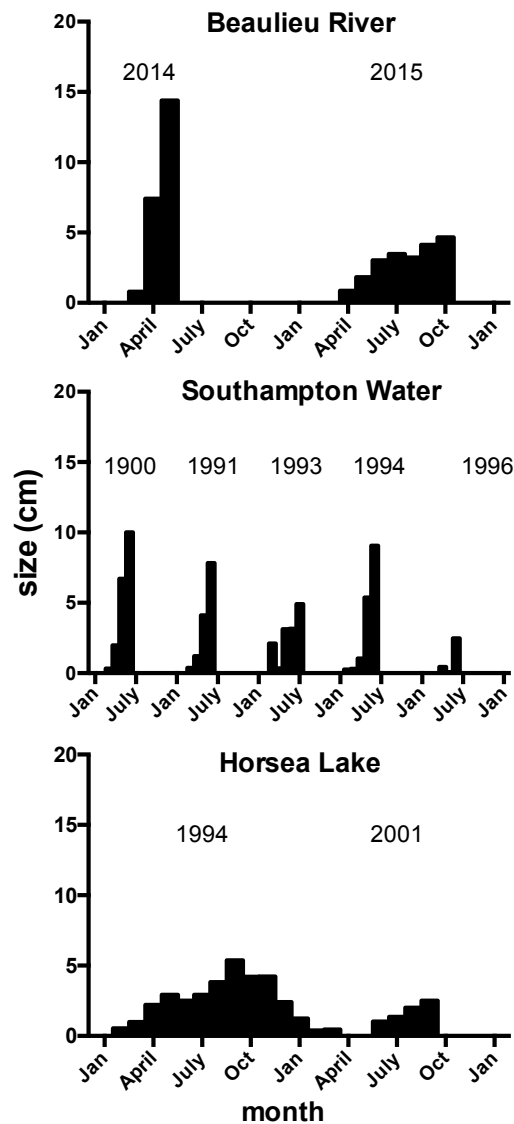


**Fig. 2.8** *Aurelia aurita* monthly size-frequency histograms from April to October 2015 and mean size $\pm$ sd (cm) versus month. Size classes scale from 0 to 7 cm and sample numbers (n) are displayed in each graph. There was a significant difference between monthly sizes (one-way ANOVA:  $F_{6,354}=99.05$ ,  $p<0.0001$ )



#### 2.4.6 Comparison of three different populations

*Aurelia aurita* sizes differed significantly between the three locations (One-way ANOVA:  $F_{2,7986}=23.41$ ,  $p<0.0001$ ), with mean Beaulieu River sizes of  $3.5 \pm 3$  cm (mean  $\pm$  sd), Southampton Water sizes  $1.3 \pm 2.3$  cm, and Horsea Lake sizes of  $1.6 \pm 1.5$  cm (Fig. 2.9). Interannual differences in sizes were observed in the Beaulieu River population (unpaired t-test:  $t=11.57$ ,  $df=33$ ,  $p<0.0001$ ), in the Horsea Lake population (unpaired t-test:  $t=42.08$ ,  $df=4915$ ,  $p<0.0001$ ) and in the Southampton Water population (One-way ANOVA:  $F_{4,2643}=14.19$ ,  $p<0.0001$ ) (Fig. 2.9).



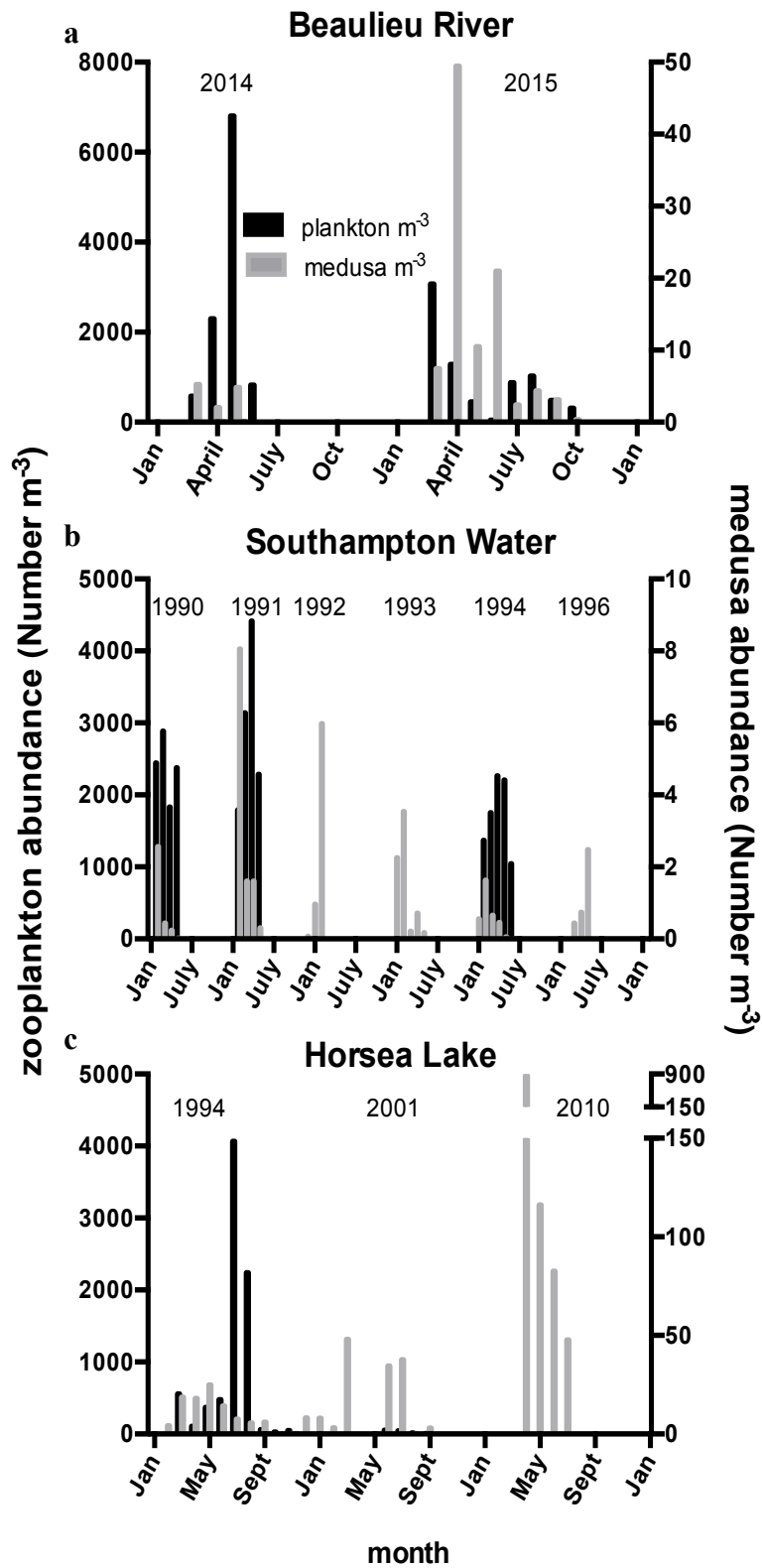
**Fig. 2.9** *Aurelia aurita* sizes over time. Bell diameters (cm) of the Beaulieu River (top) ( $n=424$ ), Southampton Water (middle) ( $n=2648$ ) and Horsea Lake (bottom) ( $n=4917$ ) population. There was a significant difference in size between the three locations (One-way ANOVA:  $F_{2,7986}=23.41$ ,  $p<0.0001$ )

Medusae abundance varied significantly between the three populations (One-way ANOVA:  $F_{2,82}=3.436$ ,  $p=0.04$ ), with mean ( $\pm$  sd) Beaulieu River abundance of  $9.9 \pm 14.75$  ind.  $m^{-3}$ , Southampton Water  $1.5 \pm 2.1$  ind.  $m^{-3}$ , and Horsea Lake  $64.2 \pm 184.8$  ind.  $m^{-3}$  (Fig. 2.10). The Beaulieu River *A. aurita* abundance did not differ significantly between the years (unpaired t-test:  $t=1.26$ ,  $df=20$ ,  $p=0.22$ ) and there were no interannual differences in the Southampton Water population (One-way ANOVA:  $F_{5,36}=1.77$ ,  $p=0.14$ ). In Horsea Lake, medusae abundance differed significantly between the years (One-way ANOVA:  $F_{2,18}=4.374$ ,  $p=0.03$ ) with an extreme high abundance of 900 ind.  $m^{-3}$  in 2010 (Fig. 2.10).

Sizes of the Southampton Water population increased with decreasing medusae abundance (Pearson  $r$ :  $r=-0.99$ ,  $p=0.03$ ), but there was no significant correlation of size and medusae abundance in the Beaulieu River population (Pearson  $r$ :  $r=-0.15$ ,  $p=0.63$ ) or in the Horsea Lake population (Pearson  $r$ :  $r=-0.41$ ,  $p=0.10$ ).

The zooplankton abundance differed significantly between the three locations (One-way ANOVA:  $F_{2,36}=8.39$ ,  $p=0.001$ ) with mean Beaulieu River abundance of  $1503 \pm 1881$  ind.  $m^{-3}$ , Southampton Water  $2646 \pm 850.3$  ind.  $m^{-3}$ , and Horsea Lake  $424.9 \pm 1022$  ind.  $m^{-3}$ .

Medusae size was significantly correlated to zooplankton abundance in the Beaulieu River (Pearson  $r$ :  $r=0.905$ ,  $p<0.001$ ), but there was no significant relationship between size and abundance of Horsea Lake medusae (Pearson  $r$ :  $r=0.18$ ,  $p=0.5$ ) and Southampton Water medusae (Pearson  $r$ :  $r=-0.17$ ,  $p=0.83$ ) (Fig. 2.9 and 2.10).



**Fig. 2.10** *Aurelia aurita* and mesozooplankton abundance over time in **a**: the Beaulieu River; **b**: Southampton Water and **c**: Horsea Lake

## 2.5 Discussion

### 2.5.1 Environmental variation in the Beaulieu River

The *Aurelia aurita* population in the Beaulieu River thrives in a variable environment, with physical factors changing rapidly on a daily and seasonal scale. The river is shallow and well mixed, with a maximum depth of 4 m at Buckler's Hard Marina. Ephyrae first appeared in the water column during February (2015) to March (2014), when temperatures were low (6°C). When temperatures were high, 24°C, in June/July, the medusae biomass was at its maximum. A similar life history pattern is found in many other temperate populations (Lucas 2001). However, the timing of *A. aurita* disappearance varied annually. In 2014, medusa disappeared in July, which is typical for the Beaulieu River (Phillips 2012). Warm summer water temperatures (24.5°C) in the Beaulieu River might have increased the metabolic rate of medusae and accelerated growth and reproductive processes but reduced life span. Advection could have been another reason for *Aurelia*'s disappearance. In 2015, summer water temperatures were about 4°C lower and jellyfish remained until October.

A similar pattern was observed in the Wadden Sea, where medusae were found to stay alive until October (Van Walraven et al. 2015). Furthermore, *A. aurita* showed a correlation between winter temperature and the first appearance of medusae and peak occurrence (Van Walraven et al. 2015). When winter water temperatures are warm, medusae appear earlier in the year as observed in Southampton Water and in the Wadden Sea (between 7 and 8°C instead of 3 and 4°C) (Lucas 2001; Van Walraven et al. 2015). It is difficult to assess the impact of winter temperature on ephyrae appearance in the Beaulieu River, as winter temperatures for 2013 are not available. It is likely that the effect of winter temperature on the reproductive activity of the polyp determines ephyrae appearance/population dynamics (Holst 2012). Experiments on

North Sea scyphozoan polyps indicates that climate warming could enhance recruitment: warmer winter temperatures might cause a longer strobilation period, a greater number of strobila per polyp, and a higher percentage of polyps strobilating (Holst 2012). However, Lynam et al. (2004) suggested that there are more jellyfish following a cold winter because this is a better trigger for strobilation. However, there is no clear pattern as the number and rate of ephyrae produced increases with food and temperature (Lucas 2001).

Oxygen concentrations declined between April and June 2014, and between March and October 2015. This is expected as warmer water holds less dissolved oxygen. *A. aurita* in the Beaulieu River was most likely not affected by a variation of oxygen concentrations, as oxygen levels were close to equilibrium and medusae are able to tolerate a range of oxygen concentrations (Arai 1997; Shoji et al. 2005). In the Black Sea planula larvae of *A. aurita* survive oxygen depleted water with less than 0.5 ml O<sub>2</sub> L<sup>-1</sup>, and medusa in the laboratory have a low DO limit of 28 ml O<sub>2</sub> L<sup>-1</sup> (Arai 1997; Shoji et al. 2005). Also polyps in Tokyo Bay are found in hypoxic conditions (Ishii and Katsukoshi 2010).

There is a great variability of salinity in the Beaulieu River, from 22 in spring to 33.5 in summer. Salinity in the Beaulieu River is influenced on the one hand by freshwater runoff from the New Forest and, on the other hand by saline coastal water inflow from the Solent. In the upper part of the estuary salinities are lower due to freshwater runoff and naturally increase towards the mouth of the estuary. *A. aurita* is euryhaline, and is found in salinities of 12 to 17 in the Baltic Sea (Kiel Bight) but also in the southern North Sea with salinities ranging from 30 to 33 (Möller 1980; Lucas 2001; Barz and Hirche 2006). The body weight of *A. aurita* increases with increasing salinities due to osmoregulation (Hirst and Lucas 1998). Furthermore, the feeding rate of *A. aurita* is

unaffected by low salinities (Hosia et al. 2012). Similarly, the polyp phase is euryhaline and can reproduce asexually at low salinities of 12 and survive in salinity of 8 (Holst and Jarms 2010). The pH in the Beaulieu River was close to neutral ranging from 7.8 to 8.8, as previously observed (Hopwood et al. 2014).

Chlorophyll *a* concentrations (indicator of phytoplankton biomass) were higher in 2014 (mean 6.56 µg/L) than in 2015 (mean 4.59 µg/L), which could have supported the higher zooplankton abundance in 2014 by bottom-up control. In general, phytoplankton levels are low in the Beaulieu River, which might be a consequence of high dissolved organic matter (DOM) that limits light penetration (Hopwood et al. 2014). The colour of the water was observed to be brown, and following high river runoff the water is very turbid with low light penetration and high particle load of 25 mg l<sup>-1</sup> suspended particle matter (Turner et al. 1998). High DOM and low light would limit phytoplankton growth or mask chlorophyll levels during analysis. Phosphate concentrations (the exclusive nutrient measured) have been observed to be sufficient for phytoplankton growth in the Beaulieu River, indicating that phytoplankton is not nutrient-limited (Phillips 2012).

#### 2.5.2 Zooplankton and *Aurelia aurita* abundance in the Beaulieu River

The seasonal cycle of *Aurelia aurita* medusae in the Beaulieu River starts with the appearance of ephyrae in February/March, which will grow and mature into medusae and reach their maximum body size in June (2014) and September (2015), before they die in October. There were interannual differences in zooplankton abundance, since the total zooplankton abundance in the Beaulieu River was five times higher in 2014 with an average of 3061.62 ind. m<sup>-3</sup> compared to 614.40 ind. m<sup>-3</sup> in 2015.

The high zooplankton abundance in 2014 coincided with low medusae abundances, which ranged from 0.26 ind. m<sup>-3</sup> to 7.55 ind. m<sup>-3</sup> and presumably caused low predation

pressure on the zooplankton population. In 2015, medusae abundance was higher, 0.29 ind. m<sup>-3</sup> to 61.18 ind. m<sup>-3</sup>, which most likely increased the predation rate on the zooplankton community. *A. aurita* size increased with increasing temperatures in the Beaulieu River. Low *A. aurita* abundance coincided with large bell diameters of 14.4 cm in 2014, and zooplankton abundance. During 2015, high *A. aurita* abundance coincided inversely with small medusa size of 4.6 cm and low food availability. Similar observations were made in the Kiel Bight where years of high medusae abundance and small bell diameters varied with years of low abundance and large bell diameters (Schneider and Behrends 1994). Medusae abundance is mainly dependent on food availability and temperature. At the end of the reproductive phase after gametes are released medusae degrade naturally (Möller 1980). *A. aurita* is known to perform vertical migration and medusae could have aggregated below the sampling depth of 1 m. Consequently, the population size might be underestimated as no vertical hauls were carried out during the sampling activity.

### 2.5.3 Comparison of three environmental different locations in the Solent

The three *Aurelia aurita* populations are closely located in the Solent and show variability in response to environmental variation. Environmental parameters of all three locations are summarised in Table 2.2 with the main differences in salinity, primary production and nutrient concentrations. The sampling time in each location varied (from two to six years), and some environmental parameters have not been recorded recently. Population dynamics of *A. aurita* are mainly driven through food resources as a function of phytoplankton/zooplankton availability and water exchange processes (Table 2.1).

**Table 2.2** Environmental parameters of the Beaulieu River, Southampton Water and Horsea Lake (NA = not available)

Environmental parameter	Beaulieu River	Reference	Southampton Water	Reference	Horsea Lake	Reference
Depth (m)	5	Höhn, present study	13		6	Lucas 1996
Temperature (°C)	3.10 - 24.60	Höhn, present study	6.20 - 22.40; 5.40 - 20.40	Lucas and Williams 1994; Muxagata et al. 2004	5.50 - 23; 5.4 - 21.8	Lucas 1996; Höhn, present study see Fig. 10
Salinity	21.80 - 33.50	Höhn, present study	29.30 - 33.50	Lucas and Williams 1994; Muxagata et al. 2004; Höhn, present study	19 - 23; 18.91 - 20.96	Lucas 1996; Höhn, present study
Chlorophyll (µg/L)	0.48 - 6.56	Höhn, present study	0.56 - 3.88; 0 - 80; 2 - 11.90	Howard et al. 1995; Hirst et al. 1999; Höhn, present study	0 - 1.20	Lucas 1996; Zampardi 2010
Oxygen concentration (%)	56.40 - 118.80	Höhn, present study	88.10 - 120.70	Höhn, present study	57.59 - 118.81	Lucas 1994, 1995, 2000, 2001
Silicate (µmol/L)	NA		1.30 - 21; 0 - 14	Howard et al. 1995; Holley et al. 2007	2.70 - 13.50	Zampardi 2010
Phosphate (µmol/L)	0.40 - 0.70	Phillips 2012	3.16 - 6.32; 0 - 0.80	Hydes et al. 2001; Holley et al. 2007	0.30 - 4.10	Zampardi 2010
Nitrate (µmol/L)	NA		0 - 3; >16.13	Hydes et al. 2001; Holley et al. 2007	NA	
Ammonium (µmol/L)	NA		0 - 7	Holley et al. 2007	2.05 - 5.47	Zampardi 2010



The zooplankton abundance in the Beaulieu River and Horsea Lake are in general low, but single high peaks during May in the Beaulieu River and August/September in Horsea Lake have occurred. The biggest *A. aurita* population of all three locations is found in Horsea Lake with exceptional high population sizes during 2010 (Fig. 2.11 c). In the Beaulieu River, the medusae abundance is greater compared to Southampton Water and showed the highest peak during April. Southampton Water has the greatest zooplankton abundance, but comprises the smallest *A. aurita* population. The mesozooplankton community in Southampton Water is dominated by cirripede nauplii and calanoid copepods (Lucas and Williams 1996). The moon jellyfish, *A. aurita*, is considered a generalist carnivore feeding on mesozooplankton and fish larvae but also on microzooplankton including ciliates and rotifers (Båmstedt 1990; Purcell 1997). Microzooplankton was missed during sampling (with a mesh size of 210 µm), and its contribution to the diet of *A. aurita* is not fully understood (Stoecker et al. 1987). The zooplankton community in Horsea Lake is species poor and mainly made up of *Acartia* calanoid copepods (*A. margalefi* and *A. tonsa*) (Lucas et al. 1997; Muxagata et al. 2004). In the Beaulieu River, the zooplankton standing stock consists of barnacle larvae, mollusc larvae and mainly copepods (present study). High nutrient levels in Southampton Water delivered by freshwater run off by the River Test and Itchen, allow high and prolonged phytoplankton blooms supporting the zooplankton stock (Kifle 1992; Lucas et al. 1997). In the Beaulieu River, high levels of sediment (turbidity) restrict phytoplankton growth and chlorophyll concentrations are much lower compared to Southampton Water (Table 2.2). The lowest phytoplankton levels of all three locations are observed in Horsea Lake (Table 2.2). Horsea Lake lacks the input of new nutrients from external sources such as the surrounding sea and rivers, as it is enclosed. However, high levels of phosphate were reported from 0.3 to 4.1 µmol/L, which could

have entered the lake by agricultural and/or local landfill contaminated ground water (Table 2.2). While phosphorus is plentiful, it could be one of the other essential nutrients that are limiting phytoplankton growth. The partially enclosed lake, Veliko Jezero, at Mljet Island in the Adriatic Sea is oligotrophic and comprises of a large *Aurelia* sp. population (10 to 600 ind. m<sup>-3</sup>) that stays in the lake all year round, similar to Horsea Lake (Colombo et al. 2009). However, Veliko Jezero has high levels of zooplankton above the thermocline (6000 to 8000 ind. m<sup>-3</sup>) and the zooplankton standing stock mainly consists of copepods similar to all three southern England locations (Colombo et al. 2009) (Table 2.2).

In general, *A. aurita* sizes increase from spring until summer when they become reproductively mature before they decrease again, due to shrinkage, and then disappear in winter similar to many temperate locations (Henroth and Gröndahl 1985; Schneider and Behrends 1994; Lucas 2001). Differences in size of *A. aurita* between the years have also been observed in the Kiel Bight and the western Wadden Sea (Van der Veer and Oorthuysen 1985; Schneider and Behrends 1998). *A. aurita* can increase in size rapidly when food is available, but can decrease in size during low food periods (Hamner and Jenssen 1974; Möller 1980). The abundance of the Horsea Lake population differed significantly between the years (Fig. 2.10). Medusae are adapted to different salinities; in Horsea Lake salinities are low between 19 and 23, whereas in Southampton Water (30 to 33) and Beaulieu River (21 to 33) salinities can be significantly higher with greater annual and daily changes. The Southampton Water medusae population showed highest abundance in March (8.71 ind. m<sup>-3</sup>) and declined rapidly afterwards until disappearance in June (Lucas and Williams 1994). However, in 1996 the highest *A. aurita* abundance was observed one month later, during June. Similarly, Raymont and Carrie (1964) reported highest *A. aurita* abundance during

May/June. Throughout the four months *A. aurita* were present, they reached a size of 12 - 14 cm in May/June, similar to Beaulieu River medusae in 2015. Growth of *A. aurita* coincided with increasing water temperature. Southampton Water has high chlorophyll concentrations of 11.3 µg/L during July, supporting the high zooplankton stock (present study). However, primary and secondary production is known to vary in Southampton Water on a spatial and temporal scale (Raymont and Carrie 1964; Hirst et al. 1999). Salinity in Southampton Water is mainly marine and changes are minimal (from 30 to 33) during the year. In the Beaulieu River, where DOM is high and chlorophyll concentrations are low 10 mg/L the highest zooplankton abundance of 7704.34 ind. m<sup>-3</sup> was observed in June 2014. One reason for high zooplankton levels in the river could be new supply by hydrodynamic mixing between the river and the Solent, or by greater predation pressure on the phytoplankton by the zooplankton. The duration of ephyrae appearance differed among the locations, with ephyrae being present for two to three months in the Beaulieu River and Southampton Water, but for seven months in Horsea Lake (Lucas and Williams 1994; Lucas 1996). The population described in the Danish fjord Kertinge Nor share many characteristics with the population in Horsea Lake (Olesen et al. 1994). At both locations strobilation begins in December and continues for seven months, medusae are very small (<50 mm) and there is a great production of individuals (25.9 ind. m<sup>-3</sup>) that remain within the habitat (Olesen et al. 1994; Riisgård et al. 2010). As found at Horsea Lake, small medusae sizes of <30 mm were present in a semi-enclosed Norwegian bay Vågsbopøllen, but *A. aurita* density was lower (22.1 ind. m<sup>3</sup>) compared to Horsea Lake and Kertinge Nor (Ishii and Båmstedt 1998; Olesen et al. 1994).

Furthermore, the three populations differ in their longevity and the Beaulieu River population for example is present until October, similar to the Wadden Sea population

(Van Walraven et al. 2015). The Southampton Water *A. aurita* population persist for four months until July, making the duration similar to *A. aurita* populations in the Gullmarfjord (Sweden) and the Fana fjord (Norway) (Henroth and Gröndahl 1985; Hosia and Båmstedt 2007). Horsea Lake medusae occur all year, but it still remains unclear if medusa live for >1 year, or if several strobilation events lead to multiple cohorts during a year (Lucas and Williams 1994; Lucas 1996). A reduction of population density in Horsea Lake from May until November suggests increased mortality and degrowth due to insufficient nutrition. A decline in population density throughout the year, has also been observed in other locations including Kertinge Nor (Goldstein and Riisgård 2016).

The medusae population has declined in Southampton Water following a survey in 2014 and recent observations (Höhn and Lucas pers obs). In the Skagerrak location, North Sea, a nine-year time series showed a similar decline of *A. aurita* from 1992 to 2011 (Hosia et al. 2014). Predation by zooplanktivores such as *Mesodinium rubrum* on planula larvae of *A. aurita* and competition for food sources (microzooplankton) could have caused a decline (Williams 1996). *A. aurita* populations could have also been influenced by the polyp phase, which strobilates in relation to seasonal conditions and produces ephyrae (Lucas et al. 2012). Changes in winter temperature may have prevented strobilation, or the polyp population could have decreased due to predation or starvation.

## 2.6 Conclusions

This investigation shows that *Aurelia aurita* populations vary between closely located locations. Each of the three locations on the south coast of England - Beaulieu River, Southampton Water and Horsea Lake is unique in its environmental parameters that coincide with the population dynamics. The physical and biological conditions in the Beaulieu River change rapidly on a daily and annual scale, and lead to interannual differences of the medusae population. Large medusae size, small abundance and high mesozooplankton levels were observed in the Beaulieu River (2014) similar to Southampton Water. Small medusae, large abundance and low levels of mesozooplankton were recorded in the Beaulieu River during 2015. Furthermore, longevity of medusae was prolonged until October during 2015, whereas in 2014 medusae disappeared after June. In contrast, Horsea Lake is a unique environment where medusae thrive all year round, whereas medusae in Southampton Water persisted for only four months in the water column - from March until June. In the Beaulieu River ephyrae appeared from March until April, which is similar to Southampton Water where ephyrae appeared during March. In contrast, Horsea Lake ephyrae are found for a prolonged time (December to June) in the water column.

Medusae growth coincides with increasing temperature in Southampton Water and with increasing mesozooplankton abundance in Southampton Water and Beaulieu River. However, the Horsea Lake population showed highest medusae abundances and lowest zooplankton abundances. In 2015, medusae abundance was high in the Beaulieu River but long-term dataset are important in determining whether an increase in jellyfish biomass does occur (Condon et al. 2012). Unfortunately, such large datasets are scarce and we still need more information about the underlying mechanisms that control bloom formation (Van Walraven et al. 2015). Jellyfish abundance decreased in some

ecosystems, but increased in other locations such as the Bering Sea, Benguela Current and Yagtzen Estuary (Mills 2001; Brodeur et al. 2002; Xian et al. 2005; Lynam et al. 2006). In Southampton Water and in the North Sea (Skagerrak) *A. aurita* abundance has declined (Hosia et al. 2014; Höhn and Lucas pers obs). More information is needed on the ecology of the polyp, which determines medusae recruitment. Therefore, polyps need to be located and observations on how their life history dynamics respond to environmental change are required.

# Chapter 3

## **Respiratory response to temperature of three populations of *Aurelia aurita* polyps in northern Europe**

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### **3. Respiratory response to temperature of three populations of *Aurelia aurita* polyps in northern Europe**

#### **3.1 Abstract**

The benthic life stage (polyp or scyphistoma) of the bloom-forming jellyfish, *Aurelia aurita* (Linnaeus 1759), also known as the moon jellyfish, contributes to the seasonal occurrence and abundance of medusa blooms via asexual reproduction. *A. aurita* is widely distributed in coastal areas in northern Europe, and one of the most studied jellyfish species. While the physiology of the visible medusa is largely understood, understanding of the perennial benthic life stage is scarce. To measure the physiological tolerance of *A. aurita*, the scyphistoma's temperature sensitivity across its distributional range was investigated. Respiration rates of polyps from three northern European locations exposed to 11 temperatures between 2 and 22°C were measured. There was a significant difference in respiration rate among the three polyp populations, which may reflect on differences in their thermal tolerance window. A critical temperature was reached at 14°C with the metabolic rate decreasing below and above that temperature. This pattern was less pronounced in the Norwegian population but polyps were able to survive, at least temporarily, those temperatures exceeding their natural range. While polyps collected from northern Norway, with a narrow environmental thermal window, displayed a low baseline metabolism with a  $Q_{10}$  value of 1.2, polyps from southern England and Scotland had  $Q_{10}$  values of 1.6 and 2.5, respectively. Differences in polyps' respiration rates across their distributional range suggest that populations have evolved adaptations to local environmental thermal conditions.



### 3.2 Introduction

Jellyfish blooms have become a concern in many regions of the world. Blooms have socio-economic impacts on fisheries and aquaculture, coastal tourism and power stations (Graham et al. 2014). Furthermore, bloom events have the potential to affect ecosystem structure and function through changes in top-down control (Schneider and Behrends 1998; Fleming et al. 2015), trophic pathways (Condon et al. 2011), and biogeochemical cycling including organic flux to the sea floor (Lebrato et al. 2011; Sweetman et al. 2015). Thus, they are the focus of concerted research efforts into understanding the causes of bloom events. The study species, *Aurelia aurita* is found in northern Europe as shown by Dawson (Dawson and Jacobs 2001; Dawson et al. 2015). *A. aurita* populations display differences in abundance and life history dynamics over a large spatial and temporal scale, which makes predictions of future bloom dynamics difficult (Lucas 2001).

The polyp life stage is critical in ensuring the successful recruitment and maintenance of jellyfish populations and, because of its ability to reproduce asexually in various ways, it is thought to be a key factor in the formation of jellyfish blooms (Lucas et al. 2012). Mechanisms and rates of asexual reproduction have been increasingly studied over the last ~10 years. Temperature is a principal environmental controller of the life cycle of scyphozoans, including survivorship, growth and reproduction in polyps (Willcox et al. 2007; Pascual et al. 2014; Schiariti et al. 2014). While it is known that temperature induces various forms of asexual reproduction such as budding, strobilation, and the formation of podocysts (Purcell et al. 2012; Han and Uye 2010; Thein et al. 2012), information about the functional biology of the polyp is largely unavailable. To my knowledge, there is one experimental study that has investigated the effect of temperature on the oxygen consumption of *Aurelia aurita* polyps in northwestern

Europe (Gambill and Peck 2014). Examining limits of temperature tolerance in polyps of bloom forming jellyfish species will help to predict the scale of recruitment of new medusa and thus the size of jellyfish populations.

While reproduction represents one way of measuring fitness, respiration provides an indirect way of measuring acute changes in metabolic rates of polyps in response to temperature variation. In ectotherms such as scyphozoan polyps, a temperature increase accelerates most physiological processes, including the rate of oxygen consumption within the temperature range an animal can tolerate. As a general rule, a rise of 10°C increases the rate of oxygen consumption by twofold to threefold which is also called the  $Q_{10}$ . Outside this normal range  $Q_{10}$  values may be less than one if temperatures are too high causing a loss of function. At low temperatures,  $Q_{10}$  values may be much larger than one possibly indicating that energy barriers and activation energy are increased (Hochachka and Somero 2002).

Living within estuarine and coastal environments, many scyphozoan medusae display high tolerance to environmental change including changes in salinity and temperature (Lucas 2001). Thermal tolerance limits within a species are the result of the environmental temperature range at a given geographic location, that can change over time and adaptation (selection for a distinct genotype) to a specific acclimation temperature is possible. However, exceptions exist and a different degree of tolerance to extreme temperatures may occur during ontogeny or certain periods of their life-cycle. As the scyphozoan *A. aurita* is widely distributed in coastal, estuarine, and brackish-water environments of northern Europe (Dawson and Jacobs 2001; Dawson et al. 2015), it is able to tolerate large and frequent temperature fluctuations typical of shallow coastal environments.

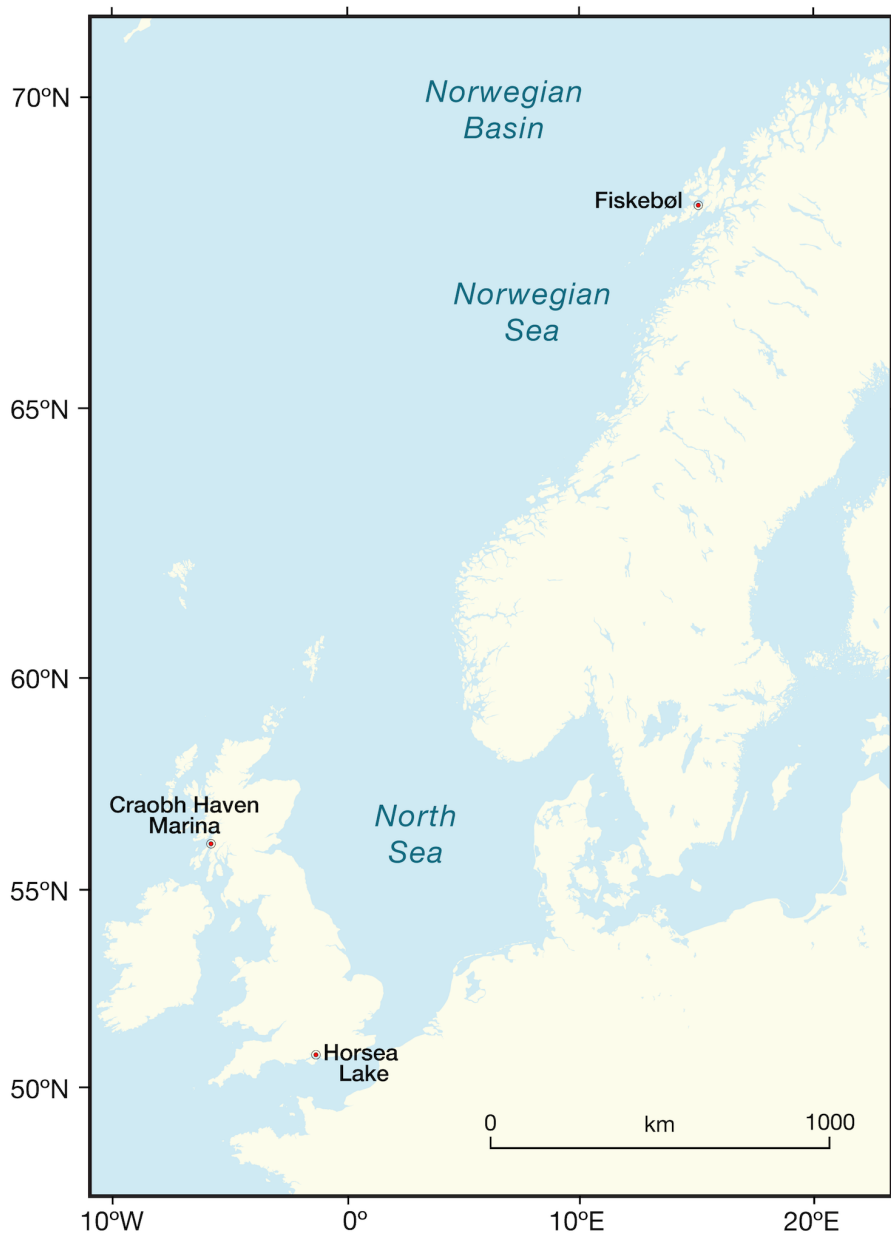
The predicted 2°C temperature increase in sea-surface temperature for the end of the 21<sup>st</sup> century associated with global warming (IPCC report 2013, RCP8.5) will expose populations to increased thermal stress, and exploring the thermal tolerance range of aquatic metazoans/jellyfish will increase the chance of realistic predictions of population size. Differences in respiration rates between geographic habitats can be used to indicate physiological tolerance in populations of *A. aurita* within northern Europe. Several studies have examined oxygen consumption in medusae, but only few have measured respiration rate in polyps (Mangum et al. 1972; Gambill and Peck 2014; Lesniowski et al. 2015).

The aim of this study is to examine the respiratory response to temperature of *A. aurita* polyps taken from three different populations within northern Europe. It is hypothesised that *A. aurita* inhabiting different geographic locations respond differently to temperatures, depending on the seasonal temperature range they experience. This will provide important insight into the metabolic rate of *A. aurita* polyps and examine the effects of latitudinal adaptation in a widely distributed coastal species.

### 3.3 Materials and methods

#### 3.3.1 Establishing polyp cultures

The following three populations of *Aurelia aurita* (Linnaeus 1759) were studied (Fig. 3.1): Horsea Lake, an enclosed brackish water lake on the south coast of England (50° 83' 68.26" N / 1° 10' 19.11" W); Craobh Haven marina, Argyll, in Scotland (56° 21' 12.79" N / 5° 55' 67.63" W); Fiskebøl, in northern Norway (68° 43' 12.92" N / 14° 82' 53.31" W). (To the best of my knowledge, all populations studied here are within species' native range (Dawson et al. 2014)).

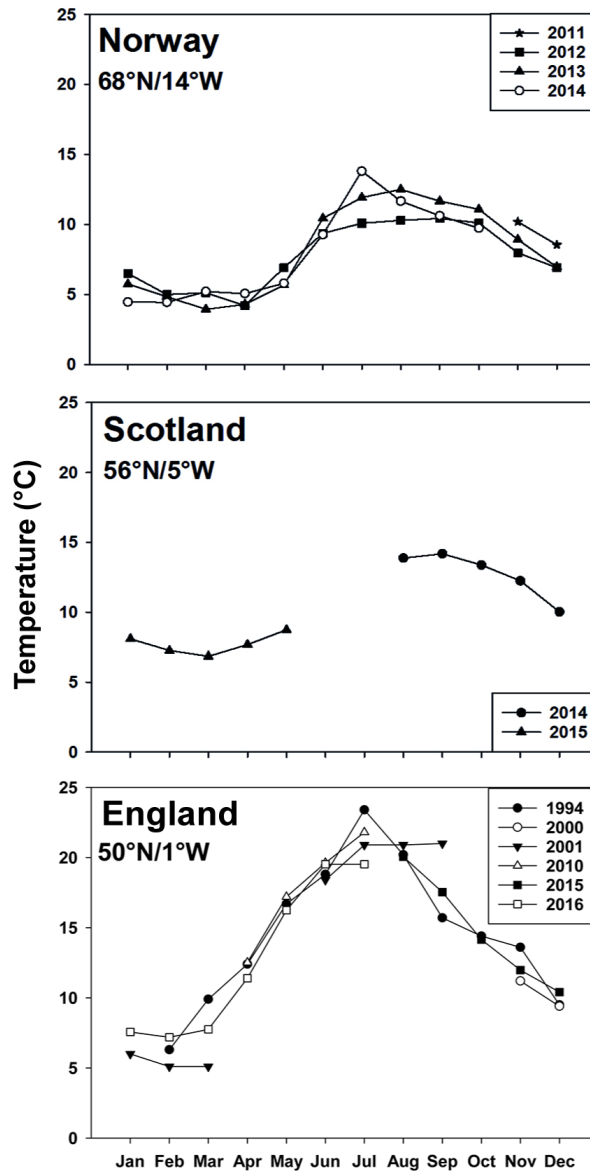


**Fig. 3.1** Map of northern Europe showing the locations of the three populations of planula-larvae bearing *Aurelia aurita* medusae that were used in this study: southern England (Horsea Lake), Scotland (Craobh Haven) and Norway (Fiskebøl)

Ripe female medusae containing planula larvae were randomly collected with a scoop net from Horsea Lake in June (Fig. 3.1). Medusae were transported back to the research aquarium at the National Oceanography Centre, Southampton (NOCS) in temperature-controlled boxes filled with ambient seawater (14°C). Oral arms of medusae were gently rubbed to release planula larvae into a small container filled with ambient

seawater (19°C), which was transported back in an insulated container to NOCS within 2 days. Petri dishes were placed on the surface of each container, for the planula larvae to settle on and metamorphose into polyps. In northern Norway ripe female *A. aurita* medusae were collected at Fiskebøl ferry terminal in June. Oral arms of medusae were gently rubbed to release planula into a small container filled with ambient seawater (14°C), which was transported back (temperature controlled) to NOCS within two days. In Scotland, ripe medusae were collected at Craobh Haven Marina, Argyll in June (14°C), and transported back to the Alan Ansell Research Aquarium at the Scottish Association for Marine Science (SAMS); larvae were settled out as described above before being transported temperature controlled to the NOCS.

When planula larvae had metamorphosed into polyps, the containers were moved into temperature controlled 12°C ± 0.5°C (salinity 32) incubators and maintained, well aerated, in darkness for four months. *A. aurita* polyps were fed two-day old *Artemia* nauplii in excess five times a week and water was changed before food was added. Polyps were exposed to eleven different temperatures, 2°C, 4°C, 6°C, 8°C, 10°C, 12°C, 14°C, 16°C, 18°C, 20°C and 22°C, capturing the full range experienced by polyp populations from southern England and exceeding the range of polyps from Scotland and Norway in their natural habitat (Fig. 3.2). Polyps experienced a temperature change of 2°C per week, starting at their acclimation temperature of 12°C. Each population was divided into two groups, with one group acclimating upwards and the second group acclimating downwards. During temperature acclimation, polyps were kept in full darkness, except for feeding and water changes (30 min d<sup>-1</sup>). Dark conditions were chosen to minimise algae growth and mimic their natural habitat. For each treatment, handling time was kept to an absolute minimum and the conditions of polyps were monitored throughout.



**Fig. 3.2** Mean monthly water temperatures at Eggum (Norway), Dunstaffnage (Scotland) and Horsea Lake (southern England) where *Aurelia aurita* were collected. Horsea Lake temperature data were taken from (Lucas et al. 1997). Norwegian temperature data were received from the Institute of Marine Research - Norwegian Marine Data Centre (NMD) and temperature data from Scotland were received from the Scottish Association for Marine Sciences (SAMS)

### 3.3.2 Respiration versus temperature experiments

Experimental polyps were last fed 24 h prior to respiration measurements, and only healthy polyps with fully developed and extended tentacles were chosen from the experimental culture. Individual polyps, of similar size, were monitored and carefully detached from each experimental container using a dissection knife before being placed

into the respiration chamber (1 polyp per chamber). Healthy polyps were released into an aquarium with 32 salinity, 1  $\mu\text{m}$ -filtered and UV treated seawater acclimated to the experimental temperature, and were then recaptured and placed in 2.8 ml plastic vials. To prevent the formation of an oxygen saturation gradient within the vial a small magnetic stirrer was placed at the bottom of each vial. A fine mesh was placed within the bottom half of the vial, for the polyp to rest on and to ensure separation of the polyp from the magnetic stirrer. The vials were sealed under water to prevent air bubbles being trapped on the inside. The sealed vials were kept upright in a water bath for the incubation period. The water bath temperature was controlled by a chiller and heater system (model: Haake DC1 K15) ( $\pm 0.5^\circ\text{C}$ ) throughout the experiment. The water bath temperature was also monitored using a thermometer placed into the water bath. The vial was monitored during the experiment, to ensure that the seawater oxygen concentration within the vial did not drop below 60% saturation, to eliminate potential effects of hypoxia (Vaquer-Sunyer and Duarte 2008). The incubation time was 4 h for each temperature treatment and experiments started at 09:00 h. Following the incubation period, the oxygen saturation of seawater inside the vial was measured using a 'Presens Microx' TX 3 temperature-adjusted oxygen meter and microoptode (Gatti et al. 2002); the microoptode was calibrated daily. The microoptode within a hypodermic needle was held in place using a clamp and stand and the optode was immediately inserted into the experimental vial once opened. Oxygen concentration ( $\mu\text{mol l}^{-1}$ ) was calculated for 100% oxygen-saturated seawater under the conditions (e.g. 32 salinity, 2.8 ml volume vial, 4 h incubation and temperature) used in the experimental treatments (Benson and Krause 1984); three controls were provided. Following the respiration experiments polyps were washed in distilled water; blotted dry on paper and were then transferred into pre-weighed 6x4 mm tin-capsules. Samples were freeze-dried for 16 h,

using a freeze drier (Thermo Scientific Heto PowerDry LL33000), and dry weights (DW) were measured using a microbalance (Sartorius ME5). To calculate the oxygen consumption of each polyp, the difference in final oxygen concentrations between the controls and experimental vials were calculated to correct for microbial respiration. Respiration rates ( $\text{nmol O}_2 \text{ h}^{-1}$ ) were calculated by including salinity, temperature, the incubation period (time in min) and the volume of the vial (see Gatti et al. 2002). For the weight specific respiration rate ( $\text{nmol O}_2 \text{ mg DW}^{-1} \text{ h}^{-1}$ ) the DW (mg) was included. The sample size of each population was: England  $n=54$ , Scotland  $n=79$  and Norway  $n=81$ . In the Scottish population one temperature is missing due to probe failure.

### 3.3.3 Statistical analyses

Statistical analysis was carried out using 'R'. An ANCOVA was performed on the respiration rates (as  $\text{nmol O}_2 \text{ h}^{-1}$ ) of the three *Aurelia aurita* polyp populations (Table 3.1). Location and water temperatures were used as factors and dry weight was used as a covariate in the model. Both factors (temperature and location) and weight showed a highly significant effect on the respiration rates ( $\text{nmol O}_2 \text{ h}^{-1}$ ) of polyps.

Mean respiration rates of the three populations were compared with a One-Way ANOVA and Holm-Sidak post hoc test.

**Table 3.1 Results of the ANCOVA.** Temperature and location were used as factors and dry weight as a covariate in the model

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
RR ( $\text{nmol O}_2 \text{ h}^{-1}$ )					
Weight	1	1316.75	1316.75	162.30	<0.0001 ***
Factor (Temperature+Location)	18	2008.00	111.56	13.75	<0.0001 ***
Residuals	112	908.64	8.11		

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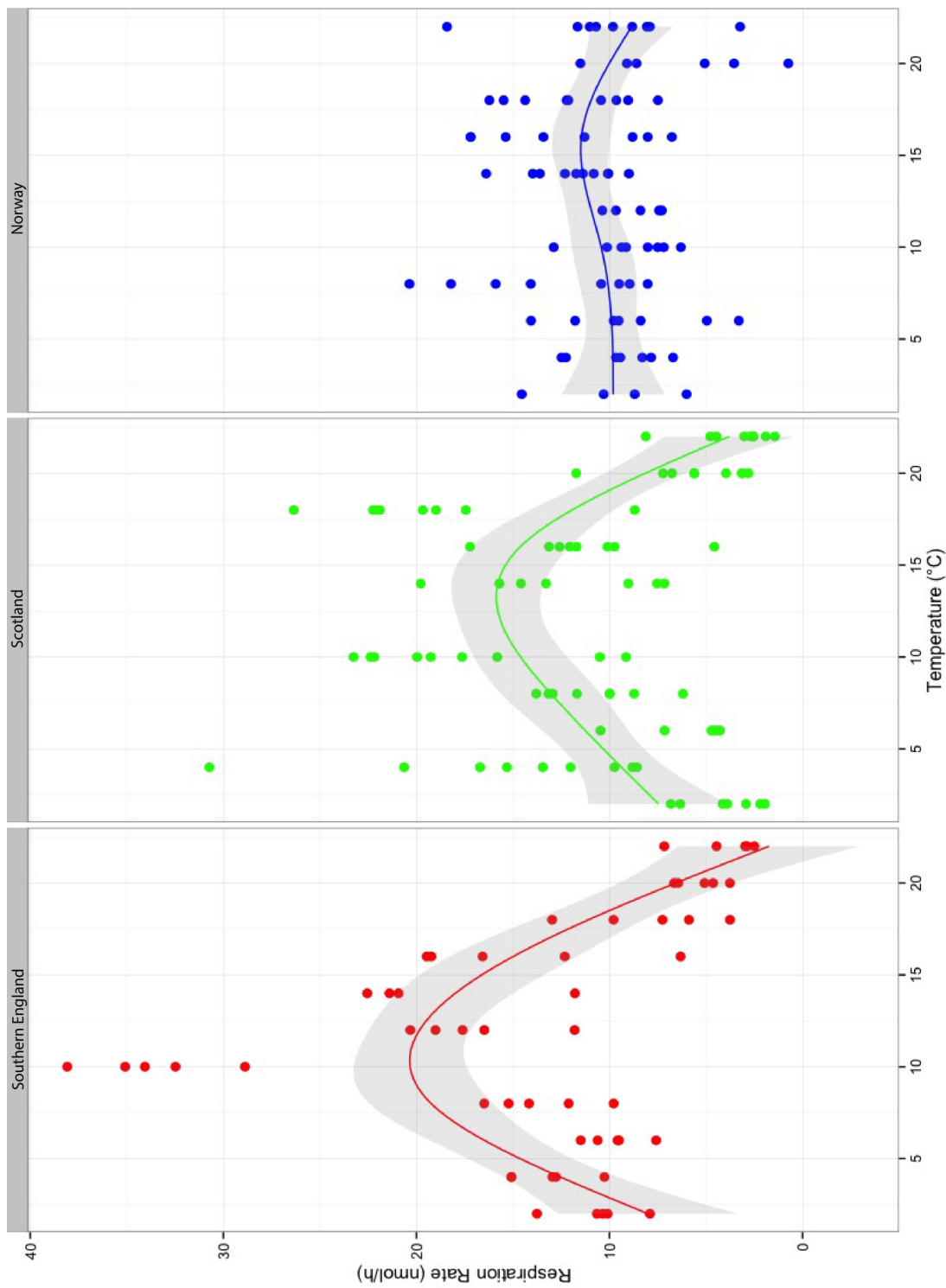


### 3.4 Results

#### 3.4.1 Respiration rates versus temperature

Overall, *Aurelia aurita* polyps' respiration rates ranged from a minimum of 1 nmol O<sub>2</sub> h<sup>-1</sup> to a maximum of 38 nmol O<sub>2</sub> h<sup>-1</sup> across the different temperatures (Fig. 3.1). Respiration rates (nmol O<sub>2</sub> h<sup>-1</sup>) increased linearly with polyp weight with a linear regression of  $R^2=0.70$  ( $P<0.05$ , see Appendix A1.1). Furthermore respiration rates were significantly different between locations and temperatures (ANCOVA:  $F_{19,112}=21.57$ ,  $P<0.0001$ ) (Table 3.1). The three *A. aurita* populations showed a different response in respiration rates over the temperature range from 2 to 22°C (One-Way ANOVA:  $F_{2,211}=4.17$ ,  $P<0.01$ ). The mean respiration rates of southern England polyps were significantly higher compared to polyps from Scotland (Holm-Sidak:  $P=0.02$ ) and Norway (Holm-Sidak:  $P=0.01$ ).

Respiration rate patterns differed among all three populations, with the greatest changes in respiration rates over temperature observed in the Scottish polyps (<1 to 36 nmol O<sub>2</sub> h<sup>-1</sup>) and in the southern England polyps (3 to 38 nmol O<sub>2</sub> h<sup>-1</sup>). The smallest changes in respiration rates were observed in the Norwegian polyps (<1 to 20 nmol O<sub>2</sub> h<sup>-1</sup>) as well as the lowest mean respiration rate (Fig. 3.3). The respiration rates of *A. aurita* polyps from southern England and Scotland increased between 2 and 14°C by Q<sub>10</sub> value of 1.6 and 2.5, respectively. The respiration rates of the Norwegian polyps increased by a Q<sub>10</sub> value of 1.2 between 2 and 14°C. After 14°C the respiration curves decreased to 22°C with a Q<sub>10</sub> value of 0.14 in the southern England and with a Q<sub>10</sub> value of 0.21 in the Scottish population. The Q<sub>10</sub> value (0.8) in the Norwegian population was similar between 14 and 22°C (Fig. 3.3).



**Fig. 3.3** Respiration rates (nmol O<sub>2</sub> h<sup>-1</sup>) of *Aurelia aurita* polyps versus temperature (2 - 22 °C). Three populations are compared: southern England (n=54), Scotland (n=79) and Norway (n=81). Scatter plot plus smoothing curve (shaded area=residuals) – a line that represents the data but does not go through each data point – is displayed. Data were analysed in R. A plot of weight-specific RR versus temperature is also available in Appendix A1.2

### 3.5 Discussion and conclusions

All organisms live within a limited range of temperatures, adjusted to all eco-physiological processes including coordination and function of molecular, cellular, and systematic cycles (Pörtner and Farrell 2008; Angilletta 2009). Thermal tolerance windows are as narrow as possible within their associated environment to minimise maintenance costs, resulting in functional differences in populations from different latitudes (Pörtner et al. 2008). The current experiment was carried out in order to examine physiological bottlenecks and differences in *Aurelia aurita* polyp populations, in terms of its respiratory response to a series of temperatures. Differences in respiratory response were observed between *A. aurita* polyps from three different geographic locations in northern Europe, indicating acclimation to local sea temperatures.

Increases in respiration rates between 2 and 14°C with  $Q_{10}$  values of 1.6 and 2.5 were observed in southern England and Scottish polyps. Similarly,  $Q_{10}$  values of 2.5 and 1.8 have been reported for Antarctic marine ectotherms and epipelagic marine zooplankton (Clarke 1983; Ikeda 1985). This indicates that the thermal effects measured here were within the species' normal range, as for many biological processes including respiration rates and enzymatic activity,  $Q_{10}$  values near two are observed (Hochachka and Somero 2002). At temperatures greater than 14°C respiration rates decreased, with  $Q_{10}$  values of 0.14 in the southern England population and 0.21 in the Scottish population, indicating that their performance had decreased rapidly after 14°C and up to a maximum temperature of 22°C, at which all biological functions ceased. Similarly, Mangum et al. (1972) observed  $Q_{10}$  values <1.00 in *A. aurita* polyps at warmer temperatures. At high temperatures lethal effects may occur with  $Q_{10}$  values less than one, indicating that increases in temperature are damaging the system in question - even leading to irreversible loss of function (Hochachka and Somero 2002).

Furthermore, the bell-shaped metabolic response to temperature could have been influenced by acclimation time (weekly temperature changes of 2°C) and duration of captivity with each 2°C change. Thermal sensitivity - the ability of an organism to withstand a range of temperatures - can be shifted by acclimation time, as well as the actual temperature. While higher reproductive rates have been observed at warmer temperatures in polyps from the Mediterranean and Baltic Sea (Purcell et al. 2012; Schiariti et al. 2014) other studies have found lower rates in polyps from Taiwan and the Mediterranean Sea (Liu et al. 2009; Prieto et al. 2010). These findings suggest that thermal optima can differ, suggesting site- and population-specific adaptation (Dam 2013).

*A. aurita* polyps collected from southern England experience a large annual temperature range from a minimum of 6°C in winter to a maximum of 23°C in summer (Fig 3.2) which might explain the large differences in respiration rates with temperature. The Scottish population experiences temperatures between 4°C and 14°C (Dunstaffnage, Fig 3.2), and this might explain the steep decline in respiration rates at temperatures above 14°C.

Gambill and Peck (2014) measured respiration rates in scyphozoan polyps from the Baltic Sea and NE Atlantic and found similar rates to our study (following conversion for comparative purposes) (Table 3.2). For this conversion, the dry weight of the Baltic Sea polyps was standardised to a dry weight at salinity 32, on the assumption that the salt content of the polyp is the same as ambient salinity (Baltic Sea, 20 salinity) (Nemazie et al. 1993).

While respiration rates of southern England and Scottish polyps peaked at 14°C, NE Atlantic polyps peaked at 12°C, and Baltic Sea polyps peaked at 15 and 18°C. All rates were lower at temperatures above and below these peaks. Temperatures >15°C are most

likely out of the normal range of Baltic Sea polyps as *in situ* temperatures from the region range between 3 and 15°C, while NE Atlantic temperatures range between 8 and 14°C (Gambill and Peck 2014). Although dry weights have been standardised, we acknowledge it might not be the best metric for characterising mass in polyps as it may change with salinity (Nemazie et al. 1993; Hirst and Lucas 1998). Nevertheless, respiration rates in both studies scaled allometrically with weight (value of 0.7 for interspecific regression) and did not increase exponentially with temperature as observed in medusae (Møller and Riisgård 2007).

**Table 3.2 *Aurelia aurita* and *Cyanea capillata* polyp oxygen consumption at different temperatures.** The respiration rates of *A. aurita* and *C. capillata* polyps from the Baltic Sea have been standardised to a DW at salinity 32, to remove the effect of salinity on weight (Nemazie et al. 1993; Hirst and Lucas 1998). Data were adapted from (Gambill and Peck 2014)

Species (µg)	Temperature (°C)	Oxygen consumption (nmol O <sub>2</sub> mg <sup>-1</sup> h <sup>-1</sup> )	Location
<i>A. aurita</i> (500)	7	5.41	Baltic Sea
	10	4.69	
	12	4.69	
	15	10.10	
	18	11.95	
	20	9.19	
<i>A. aurita</i> (525)	7	5.56	North East Atlantic
	10	5.46	
	12	13.32	
	15	11.69	

	18	12.25	
	20	6.85	
<i>C. capillata</i> (500)	7	5.95	Baltic Sea
	10	5.65	
	12	6.49	
	15	11.77	
	18	11.59	
	20	10.86	

Oxygen consumption data of polyps were derived from graphs. Data points were measured by the use of photoshop, then converted into common metrics of  $\text{nmol O}_2 \text{ mg DW}^{-1} \text{ h}^{-1}$  to be comparable with the current study. All dry weights ( $\mu\text{g}$ ) are given below each species name.

In contrast to the u-shaped response curve observed in our work and in previous studies of polyps, respiration rates in *A. aurita* medusae and ephyrae have been observed to increase exponentially with temperature. The respiration rates in *A. aurita* medusae from Skive Fjord, Denmark increased between 7 and 22°C with a higher  $Q_{10}$  value of 3.1, while the respiration rates of ephyrae increased between 11.5 and 22°C (Møller and Riisgård 2007). It seems that polyps of *A. aurita* might be less tolerant of warmer temperatures, because their respiration rates ceased at 14°C (with a lower  $Q_{10}$  value than medusae) in the present study and at 12°C in the NE Atlantic polyps (Gambill and Peck 2014). Metabolic rates among jellyfish life-stages have been suggested to scale isometrically with the lowest respiration rates in polyps compared to those of ephyrae and medusa (Glazier 2006; Møller and Riisgård 2007; Gambill and Peck 2014).

Differences are most likely caused by a different morphology, diet and activity level as polyps hardly move (Kamiyama 2013).

Norwegian polyps experience a much cooler temperature range, from 3 to 14°C, compared to those from southern England (4 - 23°C) (Fig. 3.2). The respiration rates of Norwegian polyps showed a small increase but there was little change across all measured temperatures, suggesting a lower capacity to respond physiologically to temperature variability (Pörtner et al. 2007). A low  $Q_{10}$  value in the Norwegian population may indicate that temperatures were too high, especially after 14°C, causing damage to the biochemical respiration system and leading to a loss of function (Hochachka and Somero 2002).

Overall, the three populations that were tested responded differently across the same temperature range, although all were maintained at the same conditions (12°C, 32 salinity) for about 4 months prior to the experiment. For future experiments it may be useful to measure anaerobic metabolism, as critical thermal limits are usually associated with a decrease in aerobic metabolic processes and an increase in anaerobic processes (Pörtner and Farrell 2008; Pörtner et al. 2007). Some invertebrates use metabolic depression, anaerobic energy production, and stress protection mechanism to provide short- to medium- term tolerance to extreme temperatures (Pörtner and Farrell 2008; Guppy and Withers 1999). Respiration rates differed significantly between polyps originating from higher and lower latitudes, which may be related to a variation in their thermal tolerance window, since latitudinal changes are most likely driven by environmental temperature (Pörtner and Farrell 2008). Temperatures in southern England range from 4 to 23°C, in Scotland from 4 to 14°C and in Norway from 3 to 14°C. Similarly, geographic differences in the effect of temperature on survival and asexual reproduction of *A. aurita* s.l. polyps has been observed among populations from

the Mediterranean Sea, Baltic Sea and the Red Sea (Pascual et al. 2014). The authors found that *A. aurita* polyps could maintain their reproductive features even under different environmental conditions, suggesting acclimation to their natural environment. The latitudinal differences between the three polyp populations studied here, suggest thermal adaptation to polyps' natural environment (Dam 2013). Polyps originating from higher latitudes (Scotland and Norway) experience a much narrower temperature range compared to those from southern England, but - and to our surprise - were able to maintain respiration rates at temperatures exceeding their current environmental thermal range, at least in the short term of the experiment. Nevertheless, polyps from southern England and Scotland had decreased respiration rates above 14°C indicating a sensitivity to higher temperatures. Polyps might be able to compensate in the short term for temperature changes, but differences in polyps' respiration rates across their distributional range suggests adaptation to local thermal conditions. Thus, a 2°C temperature increase in response to global greenhouse gas emission may be challenging for polyps.



# Chapter 4

**Effect of temperature on asexual reproduction of *Aurelia*  
*aurita* polyps, comparing three different populations:  
Beaulieu River, Horsea Lake and Norway**

## **4. Effect of temperature on asexual reproduction of *Aurelia aurita* polyps, comparing three different populations: Beaulieu River, Horsea Lake and Norway**

### **4.1 Abstract**

The bloom forming jellyfish *Aurelia aurita* is widely distributed in northern Europe. *A. aurita* is found in coastal, estuarine or enclosed systems and the medusa as well as the sessile polyp is exposed to thermal fluctuation. While the adult medusa is mobile and has limited ability to escape unfavourable temperatures, the perennial polyp (scyphistoma) has to withstand them. During this investigation, the reproductive performance of the polyp in response to different thermal conditions was studied. The polyp life stage reproduces asexually at different times during a year in a variety of ways. To measure *A. aurita*'s success on a latitudinal scale, scyphistomae from three environmental different locations (Beaulieu River, Horsea Lake, Norway) were cultured at three temperatures (12, 16, 18°C). The Norwegian (cold adapted) population showed the lowest survival and budding rate at 16°C and 18°C, compared to the southern England populations indicating that temperatures may have been too high. The highest budding rate was observed in Horsea Lake polyps at 16°C, followed by Beaulieu River that produced most buds at 12°C. The weight of polyps decreased with increasing temperature in Beaulieu River and Horsea Lake scyphistomae, indicating that higher metabolic demands at high temperatures could not be met. The Beaulieu River population had the highest number of cysts at 12°C and 16°C. However, the production of podocysts of the three scyphistomae populations was not different between temperatures and populations. Strobilation was not observed during the experiment, suggesting that temperatures were too high to induce transversal fission. This study

indicates reproductive variability of *A. aurita* populations. However, higher temperatures expected by global warming might be harmful for polyps.

## 4.2 Introduction

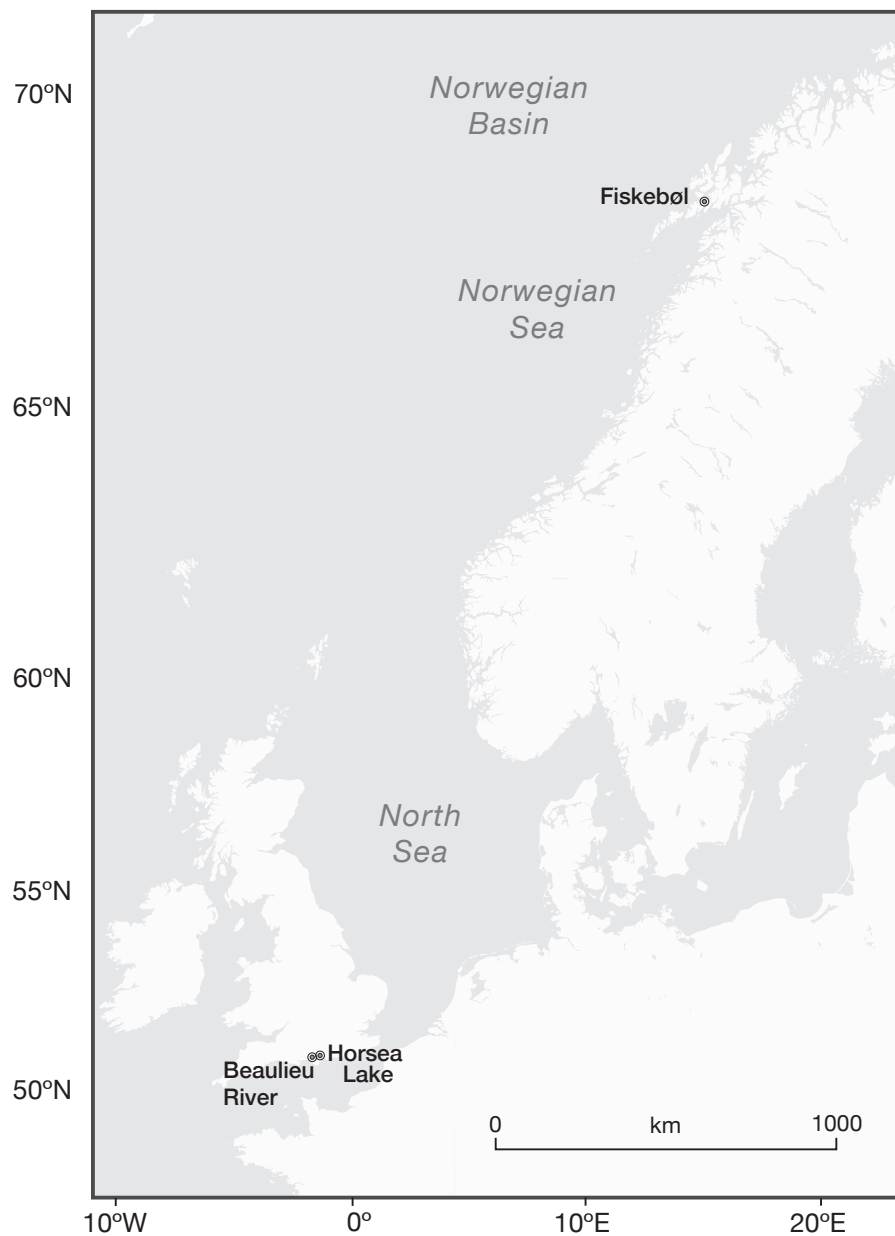
Jellyfish blooms can result in interactions with humans, including pain or even cardiac arrest from stings by their cnidocysts and cause problems for commercial fisheries by competing for the same food resources or blocking the fishing nets (Purcell et al. 2007; Pauly et al. 2009; Richardson et al. 2009; Graham et al. 2014). For these reasons jellyfish research has gained attention, and recent research efforts try to quantify population size and future bloom events (Condon et al. 2012). Anthropogenic impact such as overfishing, eutrophication, climate change and a rise of constructions that provides substrate for polyps, have been named as potential causes of increasing jellyfish numbers in some areas (Mills 2001; Purcell et al. 2007; Richardson et al. 2009). Jellyfish can outcompete fish (fishing down marine food webs) due to their high reproduction (sexual and asexual), short generation time and low carbon content (Pauly et al. 2009).

The moon jellyfish *Aurelia aurita* belongs to the order Scyphozoa and is one of the most studied jellyfish species, due to its wide distributional range and great physiological flexibility. Their ‘metagenic’ reproductive cycle is well known and *A. aurita* inhabits many contrasting environments from the English Channel to the Arctic Circle, with different environmental parameters. *A. aurita* is found in locations with different salinities: including the Baltic Sea with very low salinities (salinity 7 to 15.5) and the North Sea with high salinities (35) (Barz and Hirche 2005; Hosia et al. 2012; Holst 2012; Freund et al. 2012). Furthermore, medusae are found in areas with different temperature ranges, e.g. Scandinavian fjords with a narrow thermal range (4 to 14°C),

and in the Wadden Sea with a wider range (4.5 to 18.8°C) (Barz and Hirche 2005). *A. aurita* feed continuously on zooplankton while swimming, with a maximum growth rate of 5.7 mm d<sup>-1</sup> when food is abundant, and reach their maximum size during May/June (Möller 1980; Lucas 2001). Growth in gelatinous species might depend on food availability; when food is plentiful a medusa's bell can reach 455 mm in diameter, such as seen in the Bornholm Basin (Barz and Hirche 2005). However, when food is scarce medusae stay small <5 cm, e.g. in Horsea Lake (Lucas 1996). In the Irish Sea *A. aurita* were observed to be larger in the western Irish Sea than in the eastern part (Bastian et al. 2014). One important but often overlooked parameter in studying blooms is the ability of the perennial benthic polyp (scyphistoma) to reproduce asexually (Graham et al. 2001). The scyphistoma might be the key to jellyfish's success, due to its ability to asexually reproduce in multiple ways at different seasons. Scyphistomae's reproductive response (measure of fitness) to environmental parameters might show phenotypic plasticity. Temperature is one of the most important of environmental factors and most likely determines growth and development in polyps. In summer, polyp populations increase by budding, when temperatures are high and food is plentiful (Thiel 1962; Schiariti et al. 2014). During winter/spring, ephyrae are released by the scyphistomae (strobilation), when temperatures are at their minimum and food is scarce (Spangenberg 1965). Podocysts can also be produced by the polyp, which consists of mesoglia tissue enclosed in hard chitin cuticle, as a strategy to survive predation and periods of low food availability (Blanquet et al. 1972; Arai 2009; Thein et al. 2012). Polyps can produce multiple podocysts on stolon or pedal disc, leaving a chain of yellow beads behind, and excyst into polyps at a later stage (Black et al. 1976; Arai 2009). Only a number of studies have looked into the triggers of cyst production, and temperature changes have been named as one important parameter but this might be

population/species specific (Han and Uye 2010; Ikeda et al. 2011; Thein et al. 2012; Schiariti et al. 2014; Wang et al. 2015). Laboratory experiments on the reproduction of polyps suggest greater reproductive output in warmer temperatures, which may lead to an expansion of jellyfish numbers with global warming (Ishii and Watanabe 2003; Holst 2012; Lucas et al. 2012). In northern Europe, *A. aurita* follow a typical seasonal life cycle, and in early spring after ephyrae are produced by the polyp, they will grow into adult medusae and become reproductively mature to release planula larvae in summer (Olesen et al. 1994). Larvae will settle on the benthos and develop directly - within 10 days - into small scyphistomae (Kakinuma 1975).

During this investigation, *A. aurita* populations were collected from three different marine environments with different temperature (and salinity) conditions, including the Norwegian Sea - northern Norway (4 - 14°C), the Beaulieu River (3 - 24°C) and Horsea Lake (5 - 24°C) - southern England (Fig. 4.1). In order to determine differences among the three different environments, the effect of temperature on survival and asexual reproduction of the scyphistomae of native (Horsea Lake and Beaulieu River) and arctic (Norwegian) populations were tested. No differences in survival and asexual reproduction among populations were expected ( $H_0$ ). The effect of temperature on survival, budding rate, mode of budding, cyst production and excystment of *A. aurita* populations (Beaulieu River, Horsea Lake and Norway) were analysed under three different temperatures (12°C, 16°C and 18°C).



**Fig. 4.1** Locations of sample collection in northern Europe

### 4.3 Materials and methods

Three *Aurelia aurita* populations, maintained at the Research Aquarium of the NOC, were used in the following experiment: Horsea Lake, an enclosed lake on the south coast of England (50° 83' 68.26" N / 1° 10' 19.11" W); Beaulieu River, a small tidal river on the south coast of England (50° 80' 04.55" N / 1° 42' 28.12" W) and Fiskebøl

ferry terminal in northern Norway (68° 43' 12.92" N / 14° 82' 53.31" W) (Fig. 4.1). *Aurelia aurita* populations from areas with widely different temperature and salinity conditions were chosen. Southern England polyps were from medusae collected in Horsea Lake, which has low salinities of 19 - 21, and a wide temperature range of 5-24°C. Beaulieu River polyps originated from medusae collected from the estuary, which has wide ranging salinities of 21 - 33 and great temperature ranges of 3 - 24°C. In northern Norway - Norwegian Sea, salinity is 33 high, and temperatures are much cooler, between 4 - 14°C. Scyphistomae were cultured in the laboratory from larvae brooded by *A. aurita* medusae in their natural environment. *A. aurita* with planula larvae brooded in their oral arms were collected with a scoop net; oral arms were gently rubbed until larvae were released into containers filled with seawater. Planula larvae metamorphosed into polyps, which were maintained well aerated in 12°C 31 salinity for 5 month in dark incubators. Water was changed and scyphistomae were fed five times a week with two-day old *Artemia* nauplii.

#### 4.3.1 Experimental design

Only fully grown healthy polyps of similar size were selected prior to the experiment. Individual polyps were detached carefully and placed one-by one into a six-well plate and covered with a lid. Six replicates (one six-well plate) for each experimental treatment were provided. Polyps were left to reattach in the wells, filled with 10 mL 1-µm-filtered seawater (salinity 31), for three weeks. During the first week disturbance was kept to a minimum, and from the second week onwards *Artemia* nauplii were added daily: water of the wells was replaced with new water (with the same temperature and salinity) and new *Artemia* nauplii. Once polyps had reattached in the wells, all experimental six-well plates were placed into three dark incubators set to 12, 16 and

18°C (on the 12/11/2015). During the experiment, disturbance was kept to a minimum and six-well plates were kept in darkness except of observation periods and water changes (30 min observation<sup>-1</sup>). For each incubation temperature three replicates were used, accounting for 18 polyps per treatment. During the experiment scyphistomae were fed three times a week with two-day old *Artemia* nauplii. Seawater was changed 1h after feeding (same temperature and salinity) in order to remove debris and remaining *Artemia* larvae. One h after food was added, polyps were observed with excess of *Artemia* on their tentacles, indicating that they were fed *ad libitum*. Prior to each feeding event, the following parameters were observed: 1) specific mode of asexual reproduction, 2) number of polyps (buds) produced since the last observation, 3) the total number of reproductive products produced during the experiment. During each observation, survival was examined and newly budded (detached) polyps were removed carefully from the dish without damaging the stem polyp.

#### 4.3.2 Statistical analysis

Percentage polyp survival in all treatments was tested with a Mantel-Cox test (Table 4.1 and Fig. A2.1). Buds per polyp, cumulative buds per polyp and wet weight of polyps were tested with a two-way ANOVA, using temperature (12, 16 and 18°C) and location as factors (Beaulieu River, Horsea Lake and Norway) (Table 4.1).



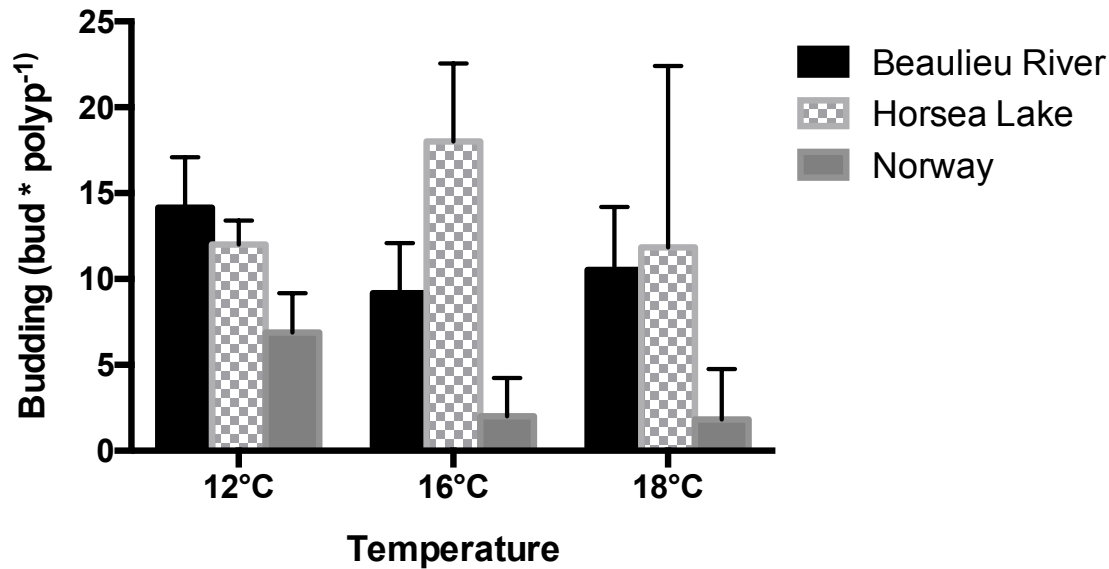
**Table 4.1** Statistical analysis of population survival at different temperatures (12°C, 16°C, 18°C) and Two-way ANOVA results showing the effect of different temperatures and location of populations on buds per polyp, cumulative buds per polyp and weight of polyps

<b>Populations</b>	<b>Temperatures</b>			<b>Statistic test</b> *=significant
<b>Survival</b>	<b>12°C</b>	<b>16°C</b>	<b>18°C</b>	<b>Mantel-Cox test</b>
Beaulieu River	6/6	6/6	5/6	$X^2=2$ , $p>0.05$
Horsea Lake	6/6	6/6	3/6	$X^2=7.3$ , $p<0.05^*$
Norway	5/6	4/6	2/6	$X^2=2.6$ , $p>0.05$
<b>Buds per polyp</b>	<b>Beaulieu River</b>	<b>Horsea Lake</b>	<b>Norway</b>	<b>Two-way ANOVA</b>
Temperature				$F_{2,2}=0.31$ , $p=0.735$
Location				$F_{2,2}=7.44$ , $p<0.001^*$
Temperature vs Location				$F_{2,4}=3.63$ , $p=0.006^*$
<b>Cumulative buds</b>	<b>Beaulieu River</b>	<b>Horsea Lake</b>	<b>Norway</b>	<b>Two-way ANOVA</b>
Temperature				$F_{2,2}=2.004$ , $p=0.139$
Location (fixed)				$F_{2,2}=13.18$ , $p<0.0001^*$
Temperature vs Location				$F_{2,4}=2.617$ , $p<0.038^*$
<b>Weight of polyps</b>	<b>Beaulieu River</b>	<b>Horsea Lake</b>	<b>Norway</b>	<b>Two-way ANOVA</b>
Temperature				$F_{2,2}=3.925$ , $p=0.028^*$
Location (fixed)				$F_{2,2}=2.172$ , $p<0.128$
Temperature vs Location				$F_{4,2}=2.519$ , $p<0.057$

## 4.4 Results

### 4.4.1 Survival of polyps at three different temperatures

Survival was high in both southern England populations, with 94.43% of Beaulieu River polyps and 83.33% of Horsea Lake polyps staying alive. However, Norwegian polyps showed a low survival rate of 61.10% combining all treatments. In all populations survival was lowest at the highest temperature, 18°C (Horsea Lake: 50%, Beaulieu River 83% and 33.30% in Norway). In Horsea Lake, survival was significantly different among the three temperatures (Mantel-Cox test:  $X^2=7.3$ ,  $p<0.05$ ) (Table 4.1).



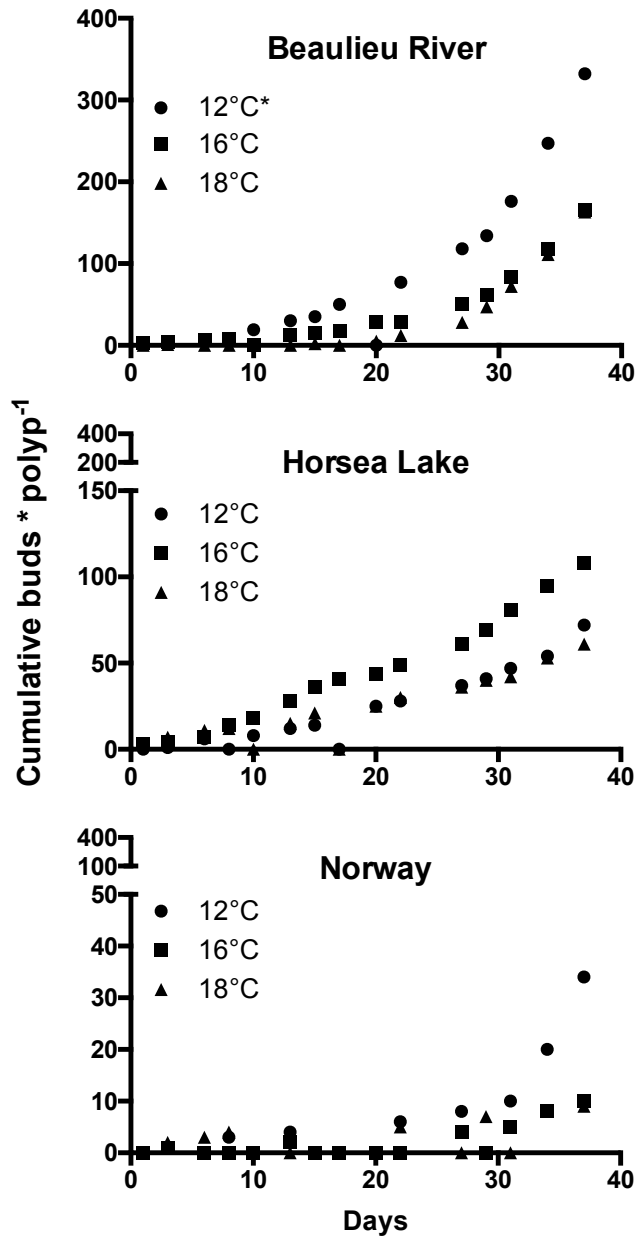
**Fig. 4.2** Total number of buds produced per polyp (mean  $\pm$  se) at the three test temperatures (12°C, 16°C, 18°C) in all populations (Beaulieu River, Horsea Lake, Norway). The location of the populations showed a significant effect on the budding rate (Two-way ANOVA:  $F_{2,2}=7.44$ ,  $p<0.001$ ) and there was a significant interaction between temperature and location ( $F_{2,4}=3.63$ ,  $p=0.006$ )

#### 4.4.2 Budding rate of polyps at three different temperatures

The number of buds per polyp differed among the three populations (Two-way ANOVA:  $F_{2,2}=7.44$ ,  $p<0.001$ ), but there was no effect of temperature on bud production (Two-way ANOVA:  $F_{2,2}=0.31$ ,  $p=0.74$ ) (Fig. 4.2). However, there was a significant interaction between temperature and location of the populations (Two-way ANOVA:  $F_{2,4}=3.63$ ,  $p=0.006$ ). At 12°C, Norway had significant lower budding rates (mean 6.89) compared to Horsea Lake (mean 12.00) (*post hoc* Holm Sidak:  $p<0.05$ ) (Table 4.1). All populations showed different budding rates at 16°C ( $p<0.05$ ), with the lowest in Norwegian polyps (mean 2.02) and the highest in Horsea Lake (mean 18.00) polyps. At 18°C, Beaulieu River polyps (mean 10.53) had significantly higher budding rates compared to the Norwegian polyps (mean 1.83) ( $p<0.05$ ). Beaulieu River polyps had significantly higher bud production at 12°C than at 16°C ( $p<0.05$ ).

#### 4.4.3 Cumulative bud production at three temperatures

There was no significant effect of temperature on the cumulative number of buds per polyp (Two-way ANOVA:  $F_{2,2}=2.004$ ,  $p=0.139$ ) (Fig. 4.3). However, the location of the populations had a significant effect on the cumulative number of buds (Two-way ANOVA:  $F_{2,2}=13.18$ ,  $p<0.0001$ ). There was a significant interaction between location and temperature (Two-way ANOVA:  $F_{2,4}=2.617$ ,  $p<0.038$ ). The Holm-Sidak *post hoc* revealed that at 12°C the Beaulieu River had significantly higher bud production over 36 days (total of 1233 buds) compared to Norwegian polyps (total 86) and Horsea Lake polyps (total 345) ( $p<0.05$ ) (Fig. 4.3 top). At 16°C, the cumulative number of buds of Norwegian polyps (total 30) was significantly lower compared to Horsea Lake (total 658) and Beaulieu River (total 605) ( $p<0.05$ ) (Table 4.1).



**Fig. 4.3** Cumulative number of buds per *Aurelia aurita* polyp over days of the experiment for each population (Beaulieu River, Horsea Lake, Norway) at each temperature (12°C, 16°C, 18°C). The location of the population had a significant effect on the cumulative number of buds per polyp (Two-way ANOVA:  $F_{2,2}=13.18$ ,  $p<0.0001$ ) and there was a significant interaction between location and temperature ( $F_{2,4}=2.617$ ,  $p<0.038$ )

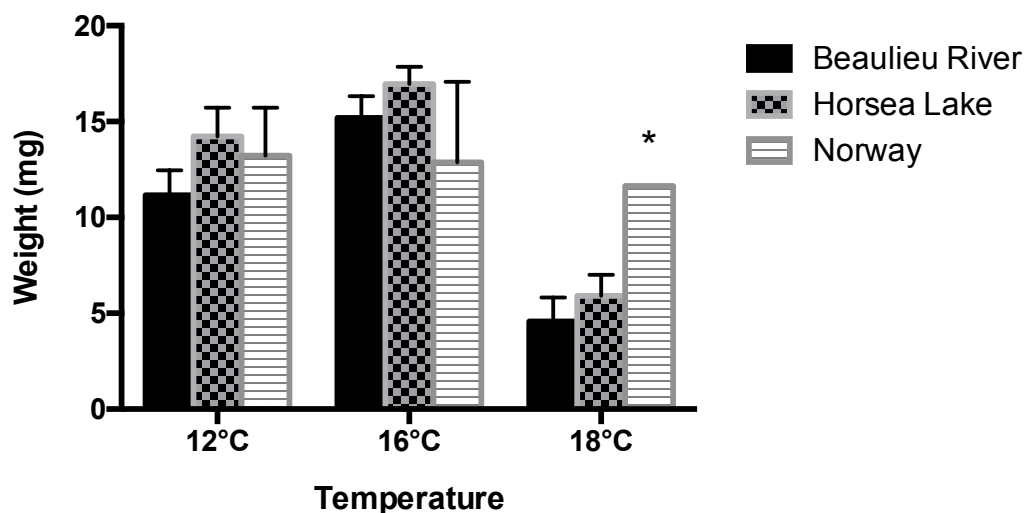
#### 4.4.4 Wet weight of polyps at all three temperatures

The wet weight of polyps was significantly affected by temperature (Two-way

ANOVA:  $F_{2,2}=3.96$ ,  $p=0.028$ ), but location had no effect on the wet weight (Two-way

ANOVA:  $F_{2,2}=2.17$ ,  $p=0.128$ ). There was no significant interaction between

temperature and location (Two-way ANOVA:  $F_{4,2}=2.52$ ,  $p<0.057$ ) (Table 4.1). Between 16°C and 18°C the wet weight differed significantly ( $p<0.05$ ). In the Beaulieu River polyps (mean 15.2 mg) and Horsea Lake polyps (mean 17 mg) maximum wet weights were observed at 16°C ( $p<0.05$ ) (Fig. 4.4). However, the lowest wet weights were observed in Norwegian polyps (mean 12.9 mg) at 16°C. In both southern England populations, the lowest wet weights were observed at 18°C ( $p<0.05$ ): mean of 4.57 mg in Beaulieu River polyps, and mean 5.91 mg in Horsea Lake polyps.



**Fig. 4.4** Wet weight of *Aurelia aurita* polyps (mean  $\pm$  se) from different populations (Beaulieu River, Horsea Lake, Norway) and at different temperatures (12°C, 16°C, 18°C). Temperature had a significant effect on the wet weight of polyps (Two-way ANOVA:  $F_{2,2}=3.96$ ,  $p=0.028$ ) and there was a significant interaction between temperature and location ( $F_{4,2}=2.52$ ,  $p<0.057$ ). \* indicates that only one sample was used

#### 4.4.5 Podocyst production during the experiment

The production of podocysts by the polyps during the experiment was low. There was no significant effect of temperature (Two-way ANOVA:  $F_{2,2}=0.492$ ,  $p=0.615$ ), location (Two-way ANOVA:  $F_{2,2}=2.336$ ,  $p=0.108$ ) or a significant interaction of both (Two-way ANOVA:  $F_{4,2}=0.246$ ,  $p=0.911$ ) on the number of cysts per polyp (Table 4.2). Beaulieu River polyps showed the highest cyst production of 0.83 at 12°C and 16°C. The lowest production of cysts was 0.17 in Horsea Lake polyps.

**Table 4.2** Mean production of cysts ( $\pm$  sd) per polyp during the experiment, at different temperatures (12°C, 16°C, 18°C) and from different locations (Beaulieu River, Horsea Lake, Norway)

Cysts per polyp (mean $\pm$ sd)	Temperatures		
Populations	12°C	16°C	18°C
Beaulieu River	0.83 $\pm$ 0.41	0.83 $\pm$ 0.98	0.50 $\pm$ 1.22
Horsea Lake	0.17 $\pm$ 0.41	0.50 $\pm$ 0.55	0.17 $\pm$ 0.41
Norway	0.33 $\pm$ 0.52	0.33 $\pm$ 0.52	0.33 $\pm$ 0.52

#### 4.5 Discussion and conclusions

The survival of *Aurelia aurita* polyps from three different environments was highest at 12 and 16°C, but low at 18°C. In Norway, the natural temperature range (4 to 14°C) is lower compared to the experimental temperatures (16 and 18°C) and was therefore most challenging for that population. Therefore, survival was greatest at 12°C and decreased with increasing temperatures. Similarly, Norwegian polyps budding rate was the lowest of all populations and decreased with increasing temperature. However, the ability to survive and continue to reproduce at a temperature exceeding 14°C (4°C higher than their natural range) shows at least temporary tolerance to higher temperatures of this cold water population.

Similar, Antarctic invertebrate species including the sea star (echinoderms) *Odontaster validus* and the limpet (molluscs) *Nacella concinna* can tolerate 4 to 5°C higher temperatures than their normal range (Peck 1989; Pörtner et al. 1999). However, this is species specific and the bivalve *Limopsis marionensis* and the scallop *Adamussium colbecki* are sensitive to only 1 to 2°C temperature increase (Pörtner et al. 1999).

Scyphozoans from the Mediterranean Sea have shown a reduction in asexual reproduction at unusual high temperatures (Purcell et al. 2012). In contrast, the bud production in Baltic Sea *A. aurita* polyps increased linearly with temperature (14, 21, 28°C) that exceeded their natural range (Pascual et al. 2014). Polyps bud new polyps

when they are not strobilating and food is plentiful, as the mother polyp and the newly budded polyp need to restore their nutrients after propagation (Thiel 1962). In northern European populations, *A. aurita* polyps produce new buds during April and August when the zooplankton stock is supported by the phytoplankton bloom (Thiel 1962; Henroth and Gröndahl 1985).

A different way of asexual reproduction was shown in the Norwegian polyps; a stolon bud production whereas Beaulieu River and Horsea Lake *A. aurita* populations displayed direct budding. Stolon bud production is linked to the production of stolons, which are outgrows from the stalk that are normally used for locomotion. Newly budded polyp will grow on the attached stolon, away from the parent polyp (Berrill 1949; Kakinuma 1975).

Stolon bud production might be a strategy to overcome intraspecific competition for space and food resources, as new substrates can be colonized and the polyp population has the potential to increase over larger spatial scale (Purcell et al. 1999; Schiariti et al. 2015). In contrast, direct budded polyps outgrow from the stalk of the parent polyp and attach next to it (Chiba 1969). On the one hand direct bud production might be of less energetic cost, as no stolon (outgrow from polyp) needs to be produced, but on the other hand the production of direct buds might be soon inhibited by high polyp density (Willcox et al. 2008; Lucas et al. 2012). Asexual reproduction (reproductive output per polyp) in *A. aurita* s.l. polyps was highest in low density populations, and lowest in high density populations, indicating density-dependent mechanisms regulating population growth (Schiariti et al. 2015). Studies on *Aurelia* polyps suggest that growth, reproduction and survival are influenced by a combination of population density and environmental conditions, which are important to the formation of jellyfish blooms (Willcox et al. 2008; Lucas et al. 2012). One of the main environmental conditions,

such as temperature and food availability affects synergistically polyps' reproduction and therefore population growth (Liu et al. 2009; Lucas et al. 2012; Schiariti et al. 2014). Typically, polyp reproduction via budding increases with temperature and food availability (Lucas 2001; Pascual et al. 2014) similar to other invertebrates (Thorson 1950; Hoegh-Gulberg and Pearse 1995). When food is scarce, usually during the winter months, energy is shifted from the production of buds towards strobilation (Wang et al. 2014). Environmental parameters such as food, temperature, salinity and photoperiod are known to synchronise annual cycles of reproduction and considered important for the reproductive output in marine invertebrates (Olive 1985). However, thermal tolerance limits in marine invertebrates are influenced by latitudinal distribution and vertical distribution on the shore and they can all experience thermal stress at their upper thermal limit likely affecting survival and reproduction.

Strobilation was not observed in *A. aurita* polyps over the duration of the experiment, as temperatures were most likely too high to induce strobilation. Strobilation usually starts during winter after a temperature minimum, of about 5°C, has been reached (Holst 2012). In Horsea Lake, polyps start to strobilate in December, when temperatures are at their minimum 6°C, and extends into June when temperatures are >12°C. However, in Baltic Sea polyps strobilation was observed at >14°C when maintained at that temperature for a long period (>180 days) (Pascual et al. 2014). However, strobilation was observed after 4 month in Horsea Lake polyps kept at 6°C, indicating the need of a low temperature over a prolonged period of time to trigger strobilation (Höhn pers obs). The production of podocysts was low in all populations (cysts produced per polyp), and there was no significant effect of temperature on the number of podocysts produced per polyp. The greatest production of podocysts was observed in the Beaulieu River population, which also had the highest survival rate, but the cyst production did not



increase with temperature. A number of studies have observed that podocyst production increases with temperature (Thein et al. 2013). In contrast, *A. aurita* polyps in the Gullmarfjord form podocysts when temperatures are at their minimum, after strobilation (Gröndahl 1988). Limited food availability might be another reason for the production of podocysts, but polyps were fed *ad libitum* with *Artemia* nauplii during the experiment (Arai 2009). The formation of podocysts might be a strategy to survive unfavourable conditions, and a number of conditions have been named to trigger podocyst formation: changes in nutrition, temperature, salinity and oxygen concentration (Holst et al. 2007; Arai 2009; Thein et al. 2013). During this study, a low number of podocysts were possibly produced alongside buds and there was no difference among the temperatures, indicating that podocysts were possibly produced as another form of reproduction in order to expand the polyp population rather than overcome unfavourable conditions. However, the changes of temperature at the beginning of the experiment might have affected the polyps in unknown ways.

The mass (weight) of polyps from the southern England populations - Beaulieu River and Horsea Lake - were lowest at 18°C. However, the Norwegian population (low number of n at 18°C) maintained a constant weight over the test temperatures. A reduction in body weight might indicate an imbalance between energy expenses for sustaining body function, as well as energy availability by nutrition. Jellyfish medusa mass (mesoglea) has been shown to decrease during low food conditions and vice versa (Lucas 2001). Furthermore, scyphozoan polyps' growth was negatively affected by poor food quality when fed P-limited copepods (Lesniewski et al. 2015). *Artemia* nauplii, which are widely used in culturing polyps, might not be a natural food source for polyps and might have affected the results in unknown ways. Due to polyps' small size their natural food sources are unknown, but microzooplankton has been named as polyps'

diet including ciliates, rotifers and also phytoplankton (Arai 1997; Raskoff et al. 2003; Zheng et al. 2012; Kamiyama 2013; Wang et al. 2015). Similarly, Han and Uye (2010) observed largest calyx diameters (somatic growth) of polyps at the lowest temperature (18°C) in comparison to the highest (28°C) temperature, indicating a reciprocal relationship between somatic growth and the production of offspring.

Comparisons among the different populations show that Horsea Lake polyps produced most buds at 16 and 18°C with 18 and 11.84 buds respectively compared to Beaulieu River and Norwegian polyps over 37 days. Scyphozoan polyps have been observed to increase budding at warmer temperatures, within their natural range (Purcell et al. 2012; Pascual et al. 2014). At 12°C, Beaulieu River polyps showed highest production of buds (higher than the other populations), decreasing at 16°C but increasing again at 18°C (lower than Horsea Lake but higher than Norway). This population was expected to be most tolerant to higher temperatures, as water temperature changes are great (11°C) in the Beaulieu River and short-term maximum summer temperatures can be high (24°C). These results imply an adaptation to great temperature changes in the Beaulieu River, and that is why polyps might have had the highest survival rate at all temperatures (94.43%). Furthermore, they displayed greatest mass (weight) at 16°C (compared to 12 and 18°C), and they might have released more buds over a longer time (exceeding the experimental time). The body weight reduction of nearly 60% at 18°C could indicate that energy was shifted from somatic growth towards budding causing a loss of polyp weight. Han and Uye (2010) observed that somatic growth of *A. aurita* s.l. and budding was negatively correlated, indicating a trade-off between energy allocation to growth and reproduction.

The budding rates differed among the populations, indicating population specific adaptation to their natural environment. While this study was conducted in the

laboratory and only temperature was considered, under natural conditions multiple parameters most likely affect reproduction. Some environmental parameters have been studied in combination, such as salinity and light, salinity and temperature, food availability and temperature, and oxygen concentration and temperature (Willcox et al. 2007; Han and Uye 2010; Thein et al. 2013; Schiariti et al. 2014; Wang et al. 2014). For example bud production increased in fed *Aurelia* sp. 1, between 10 and 15°C, to a maximum of 448 buds over 100 days (Wang et al. 2014). Willcox et al. (2007) showed that polyps of *Aurelia* sp. produced nearly twice as much buds (12) at 16°C compared to 10°C over 32 days and were not affected by different salinities. The bud production of *Aurelia* from Helgoland increased with temperature among 10, 15 and 20°C, when fed but not when starved indicating that energy need to be available for the production of new polyps (Schiariti et al. 2014).

The experiment described in this thesis was carried out on individual maintained polyps, but in the wild polyps occur in larger numbers and factors including population density, competition for food and space might limit population growth (Willcox et al. 2007; Schiariti et al. 2015). Nevertheless, the three populations from different environments retained their reproductive features even when they were under different environmental conditions.

However, a degree of genetic differentiation cannot be excluded between the Norwegian and the southern England population as genetic change in jellyfish can occur over hundreds of kilometres (Dawson et al. 2015). While in the southern England populations close genetic connections have been found (Dawson et al. 2015), no molecular studies on the Norwegian population are available.

This study suggests variability in *A. aurita* populations, and that cold adapted populations (e.g. Norway) might be at risk at warmer temperatures caused by global warming than they would experience currently in the wild.

# Chapter 5

## **Population dynamics of *Aurelia aurita* polyps in Horsea Lake: Insights into *in situ* abundance and strobilation activity**

Prepared for publication as:

Höhn DP, Lucas CH (in preparation) Population dynamics of *Aurelia aurita* polyps in Horsea Lake: Insights into *in situ* abundance and strobilation activity

## **5. Population dynamics of *Aurelia aurita* polyps in Horsea Lake:**

### **Insights into *in situ* abundance and strobilation activity**

#### **5.1 Abstract**

Natural strobilation cycles of bloom forming jellyfish polyps, which are thought to be one of the key drivers in the potential formation of jellyfish bloom events, have rarely been studied in *in situ* populations. To help address this gap, polyp population density and strobilation cycle was investigated using a photographic survey of polyps of the common jellyfish *Aurelia aurita* in their natural environment in Horsea Lake (50° 83' 68.26" N / 1° 10' 19.11" W) for 1 year between 2015 and 2016. Image analysis used to estimate polyps density over time, indicated that polyp density decreased from the summer to the winter months. Strobilation commenced in December and continued until April with peak incidence of polyp strobilation (50% of population) in January when water temperatures were at their minimum. However, the proportion of polyps strobilating was still high (20 - 40%) during December and March. Strobilation was correlated with temperature, the amount of strobilating polyps decreasing with increasing temperatures. These ecological observations agree with laboratory experiments that show that strobilation is related to temperature. A lower number of discs per strobila in comparison to laboratory polyps (i.e. 4 compared with 7) suggest a food-limited environment, as published experiments have shown that higher food availability increases ephyra production per polyp. These results confirm that strobilation in Horsea Lake occurs over a prolonged period of time, which has been observed indirectly by the presence of ephyrae in the water column in the past. I conclude that there is a highly seasonal but prolonged strobilation period in Horsea Lake and that laboratory strobilation experiments on *Artemia*-fed polyps might not reflect natural strobilation rates.

## 5.2 Introduction

The scyphozoan jellyfish *Aurelia aurita* is confined to NW Europe and is often found in enclosed or brackish water environments (Thiel 1962; Olesen et al. 1994; Lucas 1996). *A. aurita* is a bloom forming jellyfish species and blooms are formed as a result of their life cycle or by hydrodynamic processes (Graham et al. 2001; Lucas and Dawson 2014). Population variability of the visible medusa life stage has been documented in a number of studies in N Europe including Southampton Water and Horsea Lake (Lucas 1996; Lucas and Williams 1998). However, ecological knowledge about the polyp life stage is still missing in this location, as polyps are very small in size and difficult to find, located preferably underneath hard structures. The sessile polyp is a crucial part of the life cycle as the recruitment success during the polyp and ephyrae stages can have a major effect on medusa abundance, due to strobilation (the release of ephyrae). Polyp populations can expand rapidly in numbers by asexual reproduction in particular budding (producing identical polyps by lateral and stolon budding and by fission) (Schiariti et al. 2014). Budding rates have been observed to increase with increasing temperature and food availability, indicating that populations will reach their peak abundance during summer (Boreo et al. 2008). The majority of strobilation observations arise from controlled laboratory experiments using *Artemia* nauplii as polyps' diet (Thiel 1962; Holst 2012; Schiariti et al. 2015). Published natural polyp population abundance data are rare and vary greatly, due to the nature of populations often being distributed in patches and difficult to locate where medusa are found. Therefore, most previous *in situ* studies of *A. aurita* polyps have been conducted using settling plates (Henroth and Gröndahl 1983, 1985; Watanabe and Ishii 2001). Seasonal data on *in situ* *Aurelia* polyp colonies are particularly uncommon, and have only been documented in a

small number of locations – in Slovenia (Melica et al. 2014; Schiariti et al. 2015), in Tasmania (Willcox et al. 2008) and in Japan (Miyake et al. 2002; Makabe et al. 2014). The study site, Horsea Lake, provides a unique environment and is of special scientific interest, being fully enclosed from the surrounding sea developing a brackish water environment. Unusually, *Aurelia aurita* medusae are present in Horsea Lake nearly every month of the year, and do not follow a typical seasonal cycle observed in other N European locations (Lucas 1996; Lucas 2001). Ephyrae appearance is prolonged in the water column spanning up to seven months (Lucas 1996). While the pelagic medusa population was studied in Horsea Lake during part of the 1990s and 2000s (see also Chapter 2 for comparison study), the polyp populations have never been investigated quantitatively.

During this investigation, scientific divers from the University of Southampton took underwater photographs of *A. aurita* polyp populations on metal ladders in Horsea Lake. It is hypothesised that the temporal and spatial jellyfish abundance is solely dependent on environmental parameters that change within a season, such as water temperature and salinity or a combination of these. Furthermore, it is hypothesised that polyp density does not change between the months that strobilation does not change between the month and that strobilation does not change with temperature and salinity. This study will help to understand medusa recruitment patterns observed in natural populations and how they compare with laboratory observations. Here, the role that polyps play in ensuring long-term survival of jellyfish populations and the formation of blooms is studied.



### 5.3 Materials and methods

Photographs of *Aurelia aurita* polyp populations were collected by scuba diving in Horsea Lake (50° 83' 68.26" N / 1° 10' 19.11" W) between 3 and 6 m depths over 12 months, every 6 to 8 weeks (in total seven sampling events), from August 2015 to July 2016. Images were taken with a ruler (mm scale) held in place next to the polyps to calculate the size of the picture in ImageJ. After the scale of each image was determined, a 0.5 cm<sup>-2</sup> grid was drawn on the image. The amount of polyps of the same population in six random squares of 0.5 cm<sup>-2</sup> were counted in each photograph. The mean number of polyps per 0.5 cm<sup>-2</sup> were calculated and polyps per cm<sup>-2</sup> were determined.

The polyps were counted from images using 'Photoshop/ImageJ' (Abramoff et al. 2004). During these observations, strobilation was also observed and identified following Adler and Jarms (2009) and Schiariti et al. (2015). The amount of strobilating polyps, as number of strobilating polyps per cm<sup>-2</sup>, was determined like above. Then the % of polyps strobilating was calculated. Temperature data were recorded at 6 m depth, with a temperature logger taken discrete measurements every 30 minutes from August 2015 to July 2016. Salinity was measured in half-meter intervals with a hand held YSI probe.

#### 5.3.1 Statistical Analysis

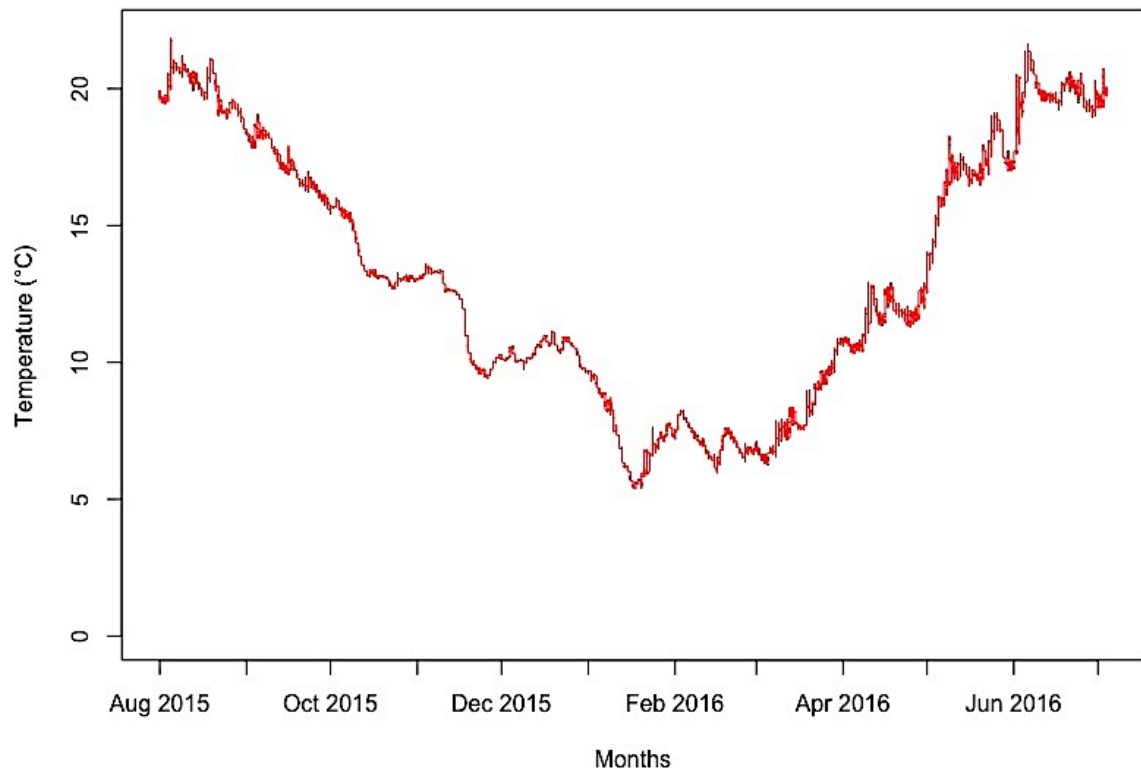
The data were analysed in the statistic program R and data of month, polyp density, strobilating polyp density, percentage of strobilating polyps, and salinity and temperature were prepared in a csv file. The effect of one categorical variable (month, salinity and temperature) was tested separately on polyp density, strobilating polyp density and percentage of strobilating polyps with a linear model (lm). The lm analyses

the relationship between the observation variable and the independent variable. When there was a statistically significant effect, a One-way ANOVA and *post hoc* Tukey test were additionally performed to examine which of the groups were significantly different from each other. ANOVA analyses the group means and it tests the hypothesis that the mean values of the measurement variables are the same in different groups. When there was a statistically significant difference between the groups ( $p < 0.05$ ), a multiple comparison method *post hoc* Tukey test was additionally conducted to determine the means which were statistically significant different from each other.

## **5.4 Results**

### **5.4.1 Environmental parameters**

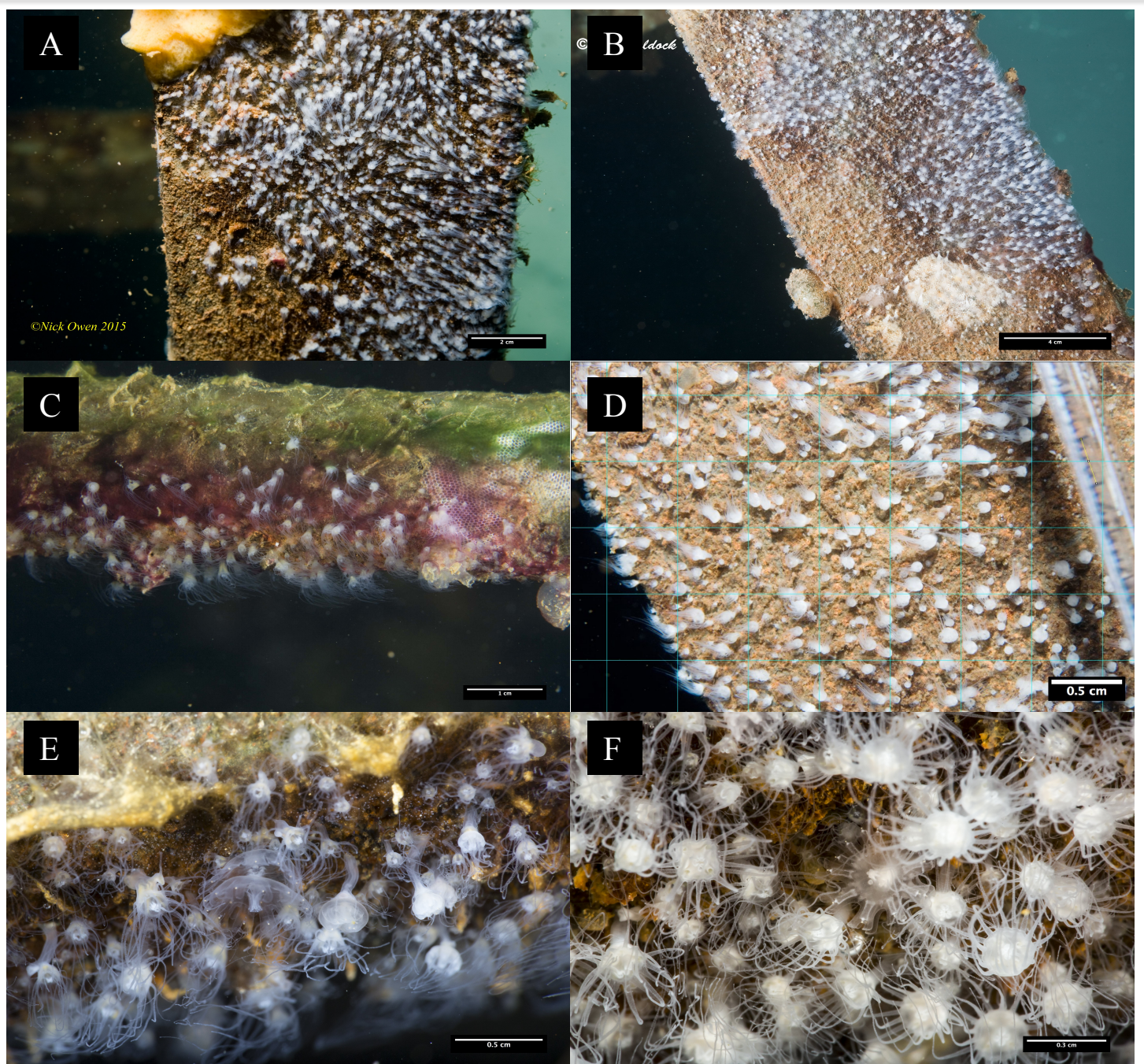
Temperature ranged from 6.70 to 21.00°C at 6 m depth (Fig. 5.1). Average monthly temperature was 20.06°C in August, 17.54°C in October, 10.40°C in December, 7.56°C in March, 11.39°C in April and 19.52°C in July. Salinity decreased from 21.00 in August to below 19.00 in April. In October salinity was 20.84, in December 20.10, in January 19.23, in March 19.04, in April 18.91 and in July 19.02. The dissolved oxygen concentration was high. The maximum value of dissolved oxygen was 14.45 mg/L and the minimum was 5.78 mg/L.



**Fig. 5.1** Temperature data from Horsea Lake at 6 m depth from August 2015 to July 2016. Measurements were taken with a temperature logger every 30 min

#### 5.4.2 Substrate

There were many fouling organisms on metal structures. Polyps were preliminary found in dense patches on the under surface of artificial metal structures, such as ladders (Fig. 5.2). The square steps of the ladders were 0.5 cm thick and 10 cm wide, and the round steps had a diameter of 2 cm. No polyps were present on the top of the step. The polyps were attached in patches. Most of the polyps were attached to bryozoans, poriferans (sponges), ascidians, *Mytilus* shells and algae mats (green and red) (Fig. 5.2 C). They also attached densely to uncovered metal substrate.

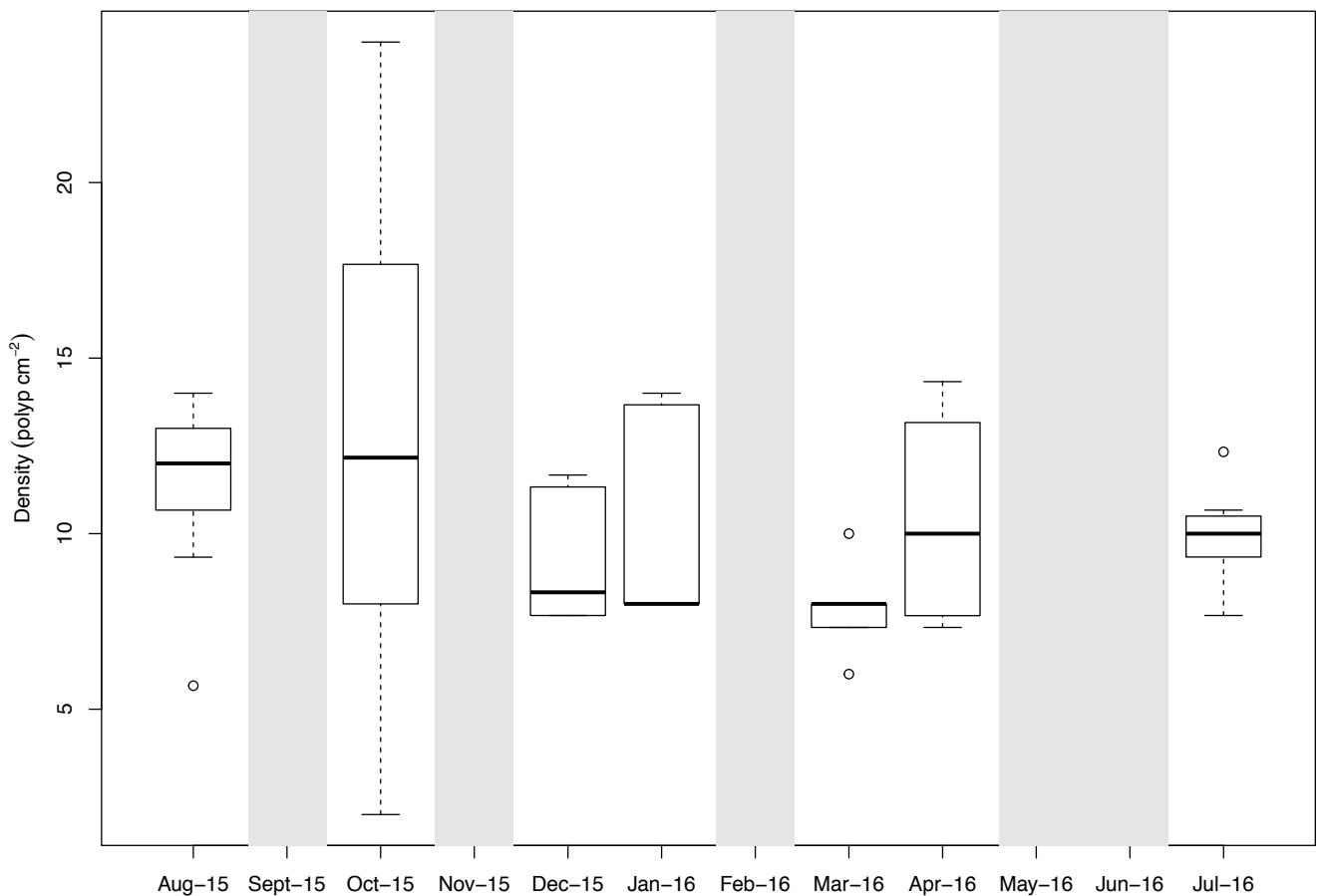


**Fig. 5.2** *In situ* pictures of *Aurelia aurita* polyp populations in Horsea Lake. A) Population before strobilation in October; B) strobilating polyps in January; C) population after strobilation in July; D) polyp population with 0.5 cm<sup>2</sup> grid; E) strobilating polyps with two discs (ephyrae) attached; F) polyp population with one disc attached. Pictures by Nick Owen, Lin Baldock and Matt Doggett



### 5.4.3 Distribution

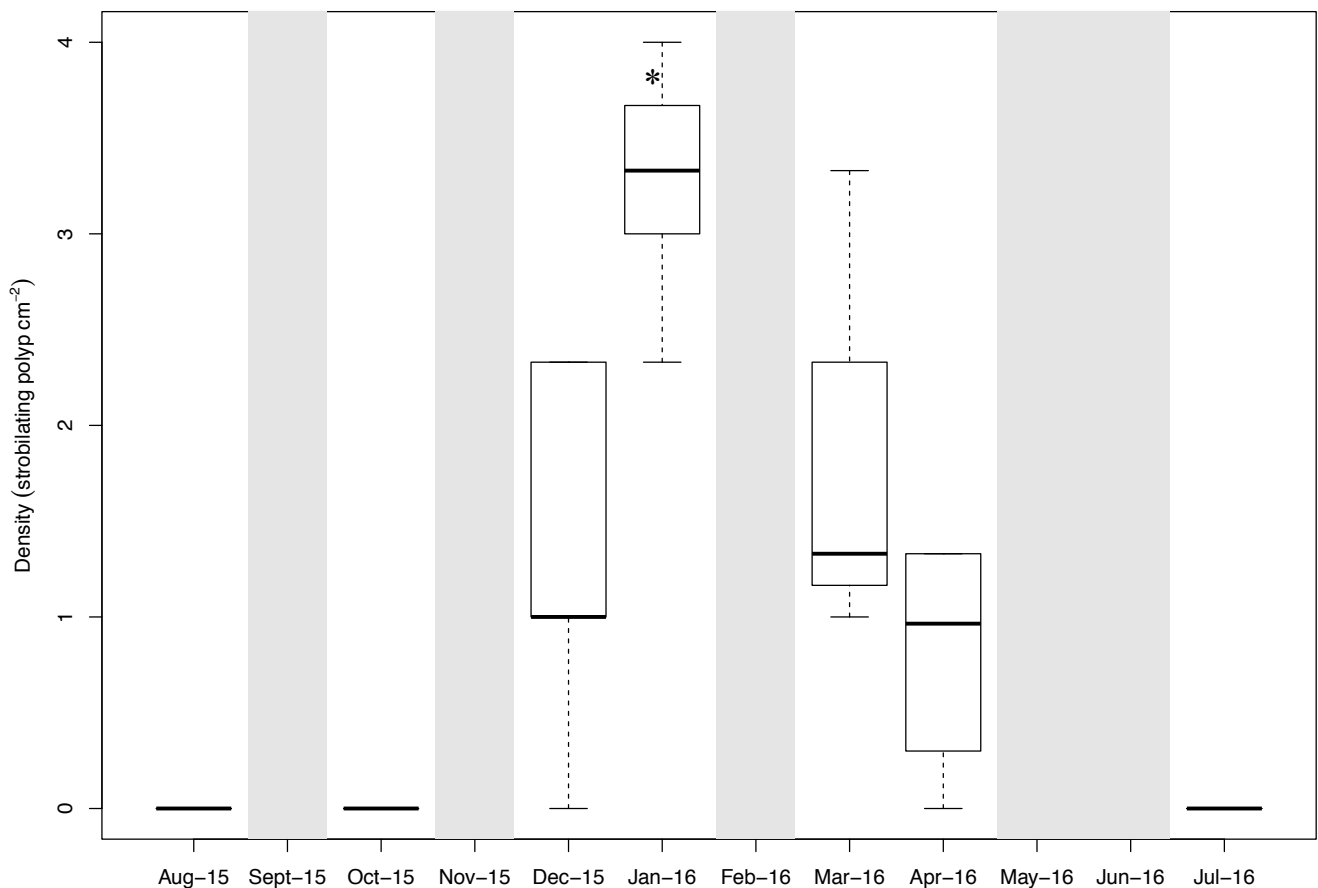
The densest part in any colony reached 32 ind. cm<sup>-2</sup> in October. The average monthly densities of polyps were 2 - 24 ind. cm<sup>-2</sup> with higher densities in summer compared to winter (Fig. 5.3). However, the density of polyps did not change significantly between the months (linear model:  $F_{1,36}=3.05$ ,  $p=0.08$ ). Similarly, temperature had no significant effect on polyp density (linear model:  $F_{1,36}=1.85$ ,  $p=0.18$ ). While lm identified a significant effect of salinity on the density (linear model:  $F_{1,36}=4.56$ ,  $p=0.04$ ), the One-Way ANOVA did not find significant differences between the months (ANOVA:  $F_{6,58}=1.18$ ,  $p=0.33$ ).



**Fig. 5.3** Density (polyp cm<sup>-2</sup>) of *Aurelia aurita* polyps over time. Density did not change significantly between the months (linear model:  $F_{1,36}=3.05$ ,  $p=0.08$ ), but in summer the density was greater compared to winter. Grey areas indicated no sampling activity

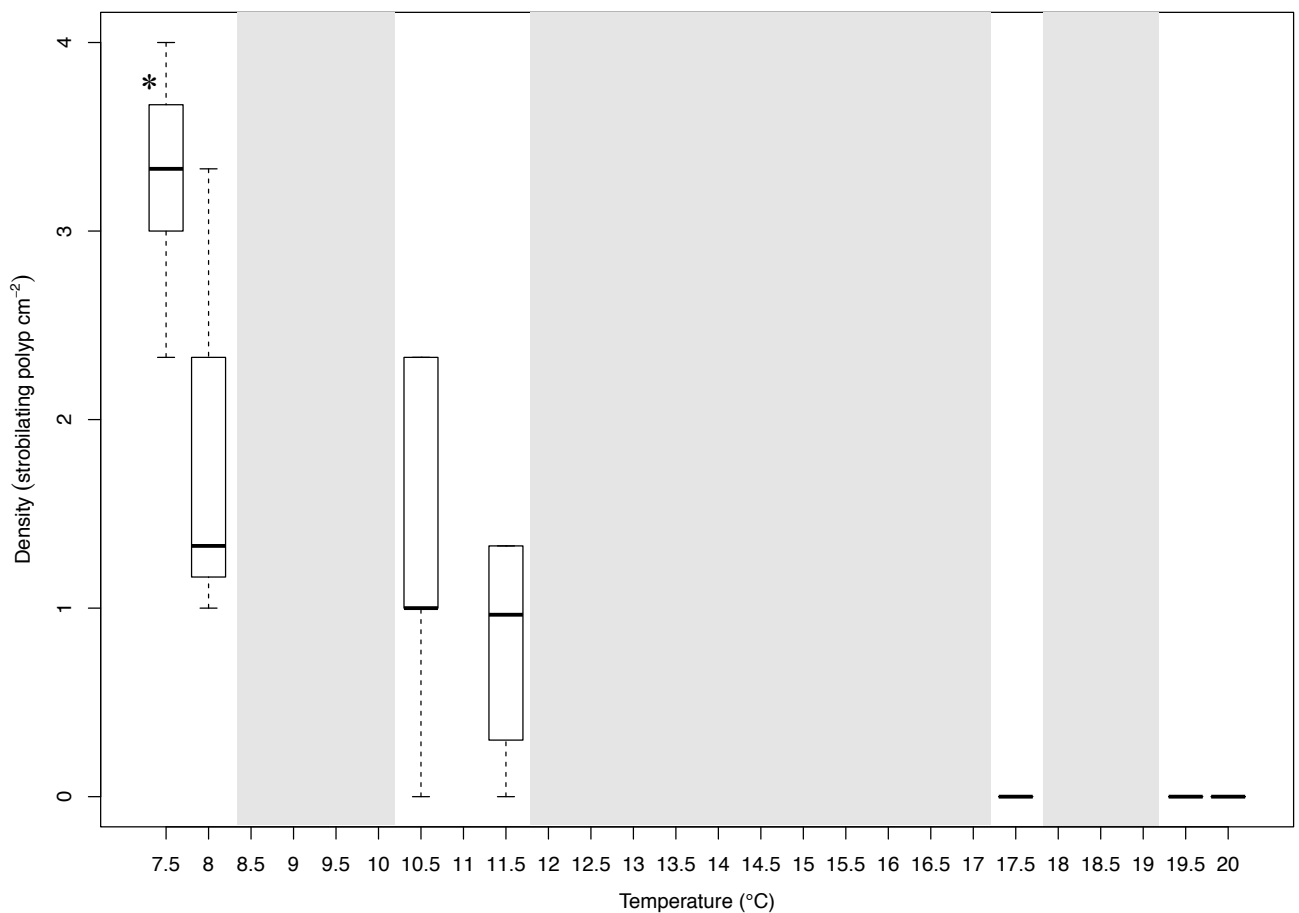
#### 5.4.4 Strobilation

Strobilation began in December when water temperatures were between 9.74 and 11.14°C with a mean of 10.40°C and continued for five months until April when mean water temperature was 11.39°C (Fig. 5.4). Transverse fission reached its peak in January with a mean of 3.3 strobilating polyps cm<sup>-2</sup>, when mean water temperature were 7.56°C. There was a significant difference in the density of strobilating polyps cm<sup>-2</sup> between the months (ANOVA:  $F_{6,57}=55.21$ ,  $p<0.0001$ ) and the Tukey *post hoc* showed that the density in January was significant higher compared to the other months: Jan vs Dec  $p<0.0001$ ; Jan vs Oct  $p<0.0001$ ; Jan vs Aug  $p<0.0001$ ; Jan vs Mar  $p<0.0001$ ; Jan vs Apr  $p<0.0001$  and Jan vs Jul  $p<0.0001$ .



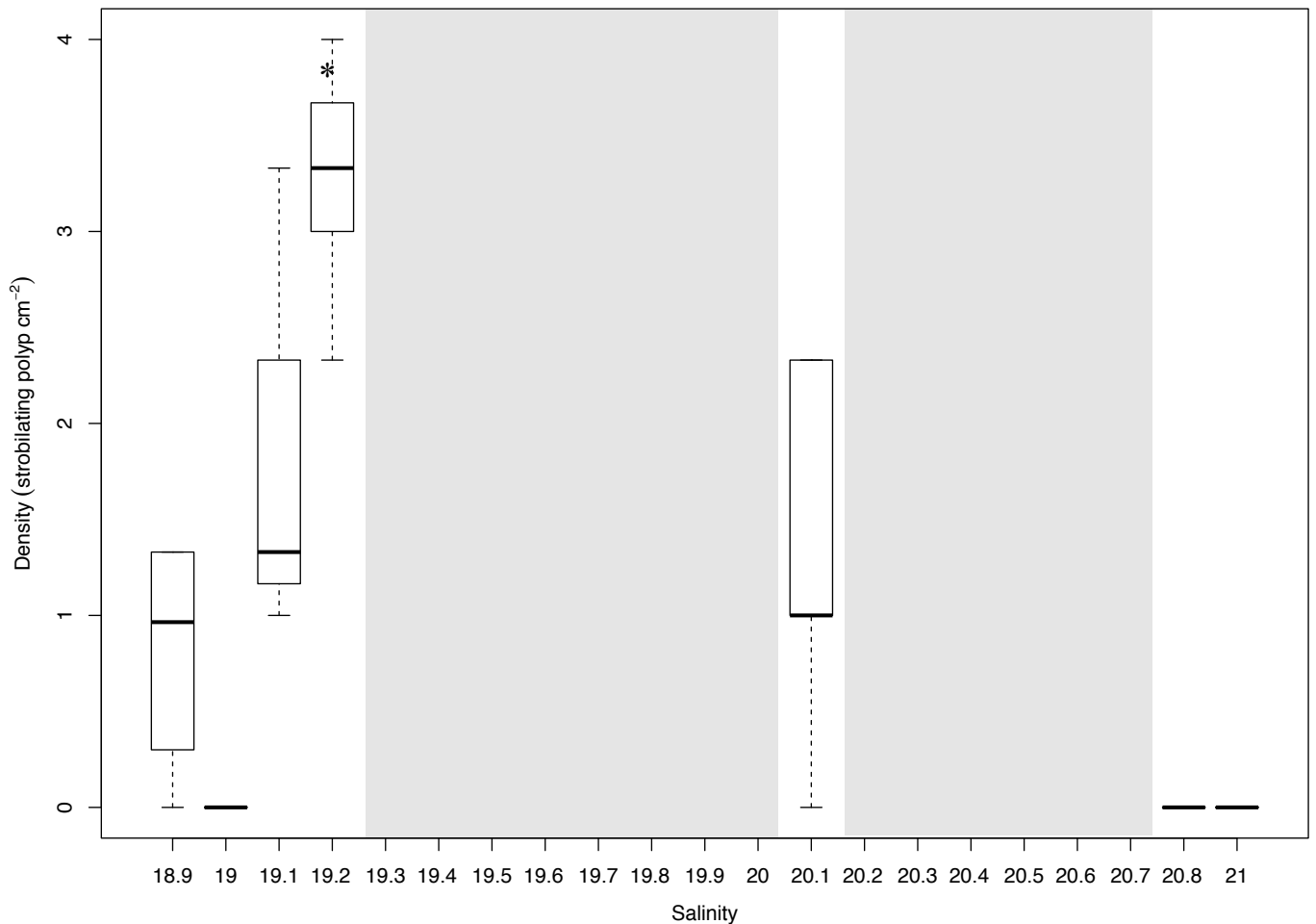
**Fig. 5.4** Density of strobilating polyps (strobilating polyp cm<sup>-2</sup>) over time. There was a significant difference in the amount of polyps strobilating between the months (ANOVA:  $F_{6,57}=55.21$ ,  $p<0.0001$ ). The greatest density of strobilating polyps was observed in January. \* indicates that January was significantly different to the other months. Grey areas indicated no sampling activity

The percentage of strobilating polyps (of total polyps) in each picture was between 13.04 and 20.56% in December, between 16.64 and 50.00% in January, between 16.63 and 41.63% in March and between 0 and 18.14% in April. Each strobila had 2 - 3 segments and the maximum number of segments was 4. There was a highly significant effect of temperature on the density of strobilating polyps  $\text{cm}^{-2}$  (linear model:  $F_{1,62}=174.3$ ,  $p<0.0001$ ) (Fig. 5.5). A multiple comparison after a One-way ANOVA (ANOVA:  $F_{6,57}=41.93$ ,  $p<0.0001$ ) revealed that strobilation was significantly higher at the lowest temperature of 7.56 °C compared to all warmer temperatures (in °C): 7.56 vs 7.74  $p=0.02$ ; 7.56 vs 11.39  $p<0.0001$ ; 7.56 vs 19.52  $p<0.0001$ ; 7.56 vs 11.39  $p<0.0001$  and 7.56 vs 19.52  $p<0.0001$  (Fig. 5.5).



**Fig. 5.5** Density of strobilating polyps (strobilating polyp  $\text{cm}^{-2}$ ) versus temperature. The amount of strobilating polyps decreased with increasing temperatures (linear model:  $F_{1,62}=174.3$ ,  $p<0.0001$ ). There was no strobilation  $>11.39^{\circ}\text{C}$ . \* indicates significant difference to temperatures  $>7.56^{\circ}\text{C}$ . Grey areas indicated no data

Salinity also had a significant effect on strobilation (linear model:  $F_{1,62}=16.44$ ,  $p<0.0001$ ) (Fig. 5.6). Strobilation started when salinity dropped from 20.96 to 20.10 and strobilation stopped after a minimum of 18.92. The highest strobilation density was reached at 19.23 (Fig. 5.6) and a multiple comparison after a One-Way ANOVA (ANOVA:  $F_{6,57}=55.21$ ,  $p<0.0001$ ) demonstrated that strobilation at 19.23 was significant higher (Tukey:  $p<0.0001$ ) compared to all other salinities: 19.23 vs 19.04  $p<0.0001$ ; 19.23 vs 18.91  $p<0.0001$ ; 19.23 vs 19.02  $p<0.0001$ ; 19.23 vs 20.84  $p<0.0001$ ; 19.23 vs 20.96  $p<0.0001$  and 19.23 vs 20.10  $p<0.0001$ .



**Fig. 5.6** Density of strobilating polyps (strobilating polyp cm<sup>-2</sup>) versus salinity. The amount of strobilating polyps increased with salinity up to 19.32 before it decreased again (linear model:  $F_{1,62}=16.44$ ,  $p<0.0001$ ). There was no strobilation  $>20.10$  salinity. \* indicates that salinity 19.23 was significant different to all salinities above and below. Grey areas indicate no data



## 5.5 Discussion

The collection of underwater photographs by scuba diving, allowed the investigation of the temporal changes in *Aurelia aurita* polyp population in Horsea Lake, as well as the timing and period of strobilation. *In situ* observations on natural substrates are rare and the production of ephyrae (strobilation) in Horsea Lake has so far only been indirectly studied by determining the presence and abundance of ephyrae in the water column (Lucas 1996).

### 5.5.1 Polyp distribution

Polyps were attached to the underside of artificial structures such as metal ladders to 6 m depth, indicating that man-made structures provide habitats for polyps (Miyake et al. 2002; Holst and Jarms 2007; Purcell et al. 2007). In areas where polyps were found many sessile organisms including bryozoans, poriferans (sponges), ascidians, mussels (*Mytilus* sp.) and algae mats were also observed. *Aurelia aurita* polyps have been observed mainly attached to mussel and oyster shells (Miyake et al. 2002; Melica et al. 2014; Schiariti et al. 2015) (Table 5.1). However, next to bivalves also algae, barnacle shells, tunics of ascidians, hydrozoans and bare rocks may be selected substrata for *Aurelia* polyps (Ussing 1927; Sjorgern 1962; Thiel 1962; Rasmussen 1973; Henroth and Gröndahl 1985). Polyps likely selected for settling places on the undersurface of metal structures, floating piers and buoys (Miyake et al. 2002). On the upper side of surfaces polyps may be eliminated by competition, predominantly algae or by light since polyps prefer shaded surfaces (Holst and Jarms 2007; Ishii and Katsukoshi 2010). The upside down position hanging from the undersurface of structures may be beneficial in additional ways; firstly no sediment is deposited on the body and secondly the polyp may be closer to resuspended food sources (Chapter 6). Resuspended

terrestrial-derived food sources may be important for polyp survival, especially during the plankton limited winter months (Höhn et al. submitted). Population size appeared greater during summer, which is likely due to greater temperature and food availability (Lucas 1996; Willcox et al. 2008; Malej et al. 2012; Höhn pers obs). Temperature and food availability seem to be one of the most important factors synergistically affecting polyp reproduction and the resulting population growth (Schiariti et al. 2015). In general, budding and consequently population growth increases with temperature and food supply in the summer months and decreases in the winter months, when temperatures drop and strobilation begins (Lucas 2001; Lucas et al. 2012; Purcell et al. 2012; Makabe et al. 2014; Pascual et al. 2014). Budding, in the form of stolonial and lateral buds, in the summer month July to October (mean temperature: 17°C) were observed to be low <5% and stopped in the winter month. Likewise, highest budding rates in polyps were observed at 16°C during laboratory experiments (Chapter 4). However, a quantitative analysis of budding was not possible due to high polyp densities masking small individuals.

Population growth may be limited by habitable space and could halt when population density becomes extremely abundant (Willcox et al. 2008; Schiariti et al. 2015). In Horsea Lake, the mean density of 3.3 polyp cm<sup>-2</sup> in January is similar to a mean density of 4.5 polyp cm<sup>-2</sup> in September on the underside of a floating pier in Hiroshima Bay, with an overall mean density of 2.6 polyp cm<sup>-2</sup> (Makabe et al. 2014) (Table 5.1). However, significantly higher *in situ* densities ranging from 6 polyp cm<sup>-2</sup> in Port of Koper (Slovenia) to 88 polyp cm<sup>-2</sup> in Kagoshima Bay (Japan) have been reported (Table 5.1). While Willcox et al. (2008) found a correlation between polyp density and rainfall (salinity) these observations have not been confirmed by laboratory experiments (Watanabe and Ishii 2001; Willcox et al. 2007). In Horsea Lake, two cohorts of polyp

sizes were observed (but did not measure them) during the summer month (August to October) possibly indicating new recruitment of polyps. This is supported by photographs of reproductive mature medusae containing planula larvae in their oral arms, taken in July.

**Table 5.1** *In situ Aurelia aurita* polyp densities (polyp cm<sup>-2</sup>) and the number of ephyrae produced by each polyp at the same time reported in the literature

Species	Location	Density (polyp/cm <sup>2</sup> )	Max. number of discs	Reference	Substrate
<i>Aurelia aurita</i>	Port of Koper, Slovenia	27	18	Malej et al. 2012	Oyster shell attached to dock pillars
<i>A. aurita</i>	Kagoshima Bay, Japan	88	14	Miyake et al. 2002	Floating piers, fouling animals
<i>A. aurita</i>	Port of Koper, Slovenia	7.78	NA	Melica et al. 2014	Oyster shell
<i>A. aurita</i>	Port of Koper, Slovenia	6	NA	Schiariti et al. 2015	Oyster shell
<i>A. aurita</i>	Hiroshima Bay, Japan	2.6	10	Makabe et al. 2014	Floating pier, metal frame, concrete surface
<i>A. aurita</i>	Horsea Lake, southern England	3.3	4	Present study	Metal ladder
<i>A. aurita</i>	Kiel Bight, Germany	NA	10	Thiel 1962	Fender
<i>A. aurita</i>	Gullmarfjord, Sweden	NA	Multiple in summer	Henroth and Gröndahl 1985	Settling plates
<i>A. aurita</i>	Tokyo Bay, Japan	NA	6	Watanabe and Ishii 2001	Settling plates
<i>A. labiata</i>	Washington, USA	9.3	10	Purcell et al. 2009	Floating docks

### 5.5.2 Strobilation

Strobilation took place in the winter months between December and April when water temperatures dropped below 12°C. Strobilation was possibly induced by a decline in temperature to 10.4°C and salinity to 20.10, but strobilation stopped when salinities decreased to 18.91. Similarly, Holst and Jarms (2010) reported that strobilation decreased with salinity from 20 with 50% to 12 with 11.6%. Similarly, bivalves have been observed to be less active in winter when salinity is low (Fisher 1988).

Decreasing temperatures have been related with strobilation in other studies (Kakinuma 1975; Miyake et al. 2002; Ishii et al. 2008; Holst 2012; Purcell et al. 2012). During laboratory experiments carried out in this thesis (Chapter 4) strobilation was observed in Horsea Lake polyps after five months when temperatures decreased from 12 to 6°C, indicating that very low temperatures may delay strobilation activity (Holst 2012; Höhn pers obs). According to Verwey (1942) a temperature between 4 and 11°C is needed for strobilation in *A. aurita* polyps. Temperature values observed to induce strobilation are likely population specific. In northern Europe, the timing of sexual and asexual reproduction in *A. aurita* changes with latitude and indicates adaptation (Lucas 2001). After autumn when food concentrations and temperature decrease, polyps may undertake strobilation and ephyrae can be observed in early spring. In Horsea Lake, the proportion of the polyp population strobilating peaked in January, 35.22%, which is higher compared to Kagoshima Bay where the incidence of strobilation peaked in January with 10.10% (Miyake et al. 2002). In Hiroshima Bay the highest fraction of strobilating polyps of 17.80% was observed in February (Makabe et al. 2014). Higher *in situ* strobilation ratios were observed in the Kiel Bight where about 85% of *A. aurita* polyps strobilated at the same time, but differed from 34 to 48% at different sites in February (Thiel 1962). *A. labiata* populations were also observed to have a high

percentage of strobilating polyps about 96% simultaneously, which differed from 13 to 42% at different sites during January to March, whereas maximum mean percentage of strobila differed from 40 to 86% (Purcell et al. 2009).

The number of *in situ* strobilae with a maximum of 4 ephyrae per polyp was low in Horsea Lake compared to 10 - 14 reported for Kagoshima Bay, Japan and up to 18 discs in the Port of Koper, Slovenia (Miyake et al. 2002; Malej et al. 2012; Makabe et al. 2014 and Table 5.1). Furthermore, greater numbers of discs were formed by *A. labiata* in Cornet Bay with 10 strobilae per polyp (Purcell et al. 2009). However, in the German Bight only 3 to 10 ephyrae were formed (Thiel 1962). A low production of ephyrae per strobilae in Horsea Lake might indicate low food availability, as food availability most likely regulates the number of discs per strobila (Lambert 1936; Thiel 1962) such as observed in laboratory experiments (Purcell et al. 1999; Gong 2001; Wang et al. 2014). However, the natural feeding ecology with its consequent effect on polyps' reproductive rate has not been studied and polyps in the laboratory are generally maintained on *Artemia* food. Polyps maintained in the laboratory produced a greater number of discs per strobila of 7 during strobilation (Höhn pers obs). Similarly, Holst (2012) observed 7 discs on North Sea *A. aurita* polyps after a temperature change of 15-10-15°C when fed weekly *Artemia* nauplii, but results from laboratory experiments might differ from field observations (Purcell et al. 2009). Furthermore, low food availability has previously been reported to be the cause of small medusa bell sizes in Horsea Lake (Lucas 1996). Nevertheless, temperature has been named as the main environmental parameter affecting strobilation in jellyfish polyps (Purcell et al. 2009), thus a combination of food and temperature might synergistically affect strobilation and the number of medusa produced. Salinity may also have an effect on strobilation as Holst and Jarms (2010)

observed a higher production of discs by *A. aurita* at high salinities (28, 36) compared to a very low salinity (12).

The duration of strobilation of about seven months in Horsea Lake is prolonged compared to three months in closely located populations of Southampton Water and Beaulieu River (Lucas 1996; Lucas and Williams 1994; Chapter 2; Höhn pers obs). Similarly, Makabe et al. (2014) reported a strobilation period from December to April in *A. aurita* in Hiroshima Bay and Olesen et al. (1994) observed strobilation from December for 7 month onwards in the shallow Danish Fjord, Kertinge Nor. Prolonged strobilation in closed or semi- enclosed lakes or fjords may be due to minor physical disturbance (no tidal flushing), low salinity between 15 and 21 in Kertinge Nor (Riisgård et al. 2008) and between 18 and 21 in Horsea Lake (present study) or due to environmental factors regulating the density of populations (Willcox et al. 2008). Protracted life histories spreading over many months, may suggest relaxed selection of synchronized strobilation and ontogeny since medusa will have a reduced advection in an enclosed small area such as seen in other lakes (Dawson and Martin 2001). Semi-continuous strobilation might reflect relaxed predation pressure by medusae on the zooplankton stock owing to the food-limited environment Horsea Lake displays. Warmer mean winter temperatures of 10 - 12°C in southern England might result in a prolonged strobilation phase in *A. aurita*, as observed in North Sea polyps by Holst (2012) in the laboratory.

## 5.6 Conclusions

Prolonged duration of strobilation activity was observed in Horsea Lake *Aurelia aurita* polyps, with the period of ephyra production lasting from December to April confirming past observations of ephyrae present in the water column (Lucas 1996). The

proportion of strobilating polyps was significantly related to water temperature and salinity. Decreasing water temperatures in autumn induced strobilation, as observed by numerous laboratory studies. The small number of strobila of 4 per polyp could have been caused by low food availability.

While the density of polyps does not change significantly over time, there was a clear trend of greater densities during the summer month and decreasing densities during the winter month. Budding was only observed during the summer month (July to October). Population growth including budding might depend on increasing temperatures and food availability typically for the summer month. I conclude that strobilation rates observed during laboratory experiments on *Artemia*-fed polyps might not reflect natural rates.





# Chapter 6

**Insights into the feeding and metabolism of jellyfish polyps in  
wild and laboratory conditions: do experiments overestimate  
natural functional rates?**

Prepared for publication as:

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natural functional rates? *Marine Biology*

## **6. Insights into the feeding and metabolism of jellyfish polyps in wild and laboratory conditions: do experiments overestimate natural functional rates?**

### **6.1 Abstract**

Gelatinous zooplankton makes a major contribution to the total biomass in the ocean and recently the incidence or size of jellyfish blooms have been observed to increase in several regions of the world. The feeding and reproductive ecology of the small scyphozoan polyp life stage is thought to be a major driver of jellyfish blooms, but polyp ecology is rarely studied under field conditions. Here, stable isotopes of carbon and nitrogen were used to investigate feeding ecology in *Aurelia aurita* polyps from the Beaulieu River (50° 80' 04.55" N / 1° 42' 28.12" W) in both winter and summer conditions, and compared to laboratory maintained polyps fed *Artemia* nauplii at 6°C and 20°C. In natural conditions, *A. aurita* polyps were predominantly supported by benthic food pathways (bacteria, detritivores, epibenthic organisms), with the primary carbon source changing from a terrestrial plant-derived source in winter towards a phytoplankton or sediment-derived source in summer. Polyps in the laboratory assimilated *Artemia* food at 6°C although metabolic processes were reduced, while at 20°C, polyps starved as their increased metabolic costs could not be met from the *Artemia* food. These ecological findings suggest that wild *A. aurita* polyps living in estuaries such as the Beaulieu river are not primarily supported by the zooplankton prey that are commonly offered in laboratory experiments. Experiments on growth and asexual reproduction of *Artemia*-fed polyps might not reflect natural metabolic rates especially at warmer temperatures (e.g. 20°C), because these polyps are not extracting sufficient resources from their *Artemia* food to fuel the increased metabolic costs.

## 6.2 Introduction

Over the last 10 - 15 years, jellyfish blooms have become a concern in many regions of the world, impacting socio-economic activities and influencing marine ecosystem structure and function through top-down predatory control over zooplankton populations (Richardson et al. 2009; Graham et al. 2014). We use the term jellyfish as a synonym for members of the gelatinous zooplankton belonging to the phyla Cnidaria and Ctenophora. Jellyfish bloom events have the potential to affect pelagic fisheries, biogeochemical cycling and organic flux to the seafloor, and are thus the focus of concerted research efforts into understanding the causes of bloom events (Condon et al. 2012; Fleming et al. 2015; Sweetman et al. 2015). Jellyfish make a major contribution to the total biomass in the ocean, but it is difficult to accurately define their trophic role, thus limiting our understanding of marine food webs in general (Pauly et al. 2009; Lucas et al. 2014).

The complex life cycle associated with most bloom-forming species including the common scyphomedusa *Aurelia aurita* makes it extremely difficult to understand what is important in driving their population abundance and phenology. As with most scyphozoans, *A. aurita* is characterised by a metagenic life cycle alternating between an asexual reproducing benthic polyp and a sexual reproducing medusa (Arai 1997; Hamner and Dawson 2009). The polyp is able to produce new polyps (via budding), and following the sea-temperature minimum, juvenile planktonic ephyrae are produced by strobilation, which grow and mature into adult medusae (Lucas 2001). While the polyp life stage is critical in ensuring the successful recruitment and maintenance of medusae populations, it is also thought to be a key factor in the formation of problematic jellyfish blooms (Prieto et al. 2010). Despite the increase of scientific papers on jellyfish blooms in the last decade, ecological/physiological and bioenergetic studies remain poorly

combined. Consequently, more information is required on how factors such as abiotic/biotic environmental characteristics and functional biology interact to promote jellyfish blooms, preferably in each life stage.

In comparison with the well-studied medusa life stage, little is known about polyp biology and ecology, particularly in *in situ* populations (Purcell et al. 2007; Holst 2012). The benthic polyp life stage is of critical interest as asexual reproduction enables bloom formation by increasing the source (polyps) and supply (ephyrae) of new recruits to the medusa population (Lucas and Dawson 2014). Information about the functional biology of the benthic life history of the polyp, and how this translates into somatic and asexual reproductive growth, and ultimately blooms, is limited, but is required to help predict the scale of medusa populations between years and locations (Lucas et al. 2012; Gambill and Peck 2014).

Laboratory-reared polyps used for measurements of growth and asexual reproduction are considered to be carnivorous, like the medusa life stage, and are successfully and regularly maintained on cultures of *Artemia* nauplii (brine shrimp) (Schiariti et al. 2008; Pascual et al. 2014). Because of their small size and the unsuitability of methods such as gut content analysis and lab clearance rates, there is virtually no information about polyp diet, feeding rates or metabolism under natural conditions (Wang et al. 2015). Unlike the seasonally occurring pelagic medusa life stage, the polyp is present year-round, and is therefore likely to experience a wide range of temperature and food conditions. Jellyfish typically inhabit coastal seas and estuaries, where a wide variety of food sources such as zooplankton (Purcell 1992), bacteria, and terrestrial- or marine-derived detritus are available. While it is possible that during spring and summer polyps feed on micro- and meso-zooplankton (Kamiyama 2011, 2013), these food resources

become scarce in the winter months in temperate regions, and alternative diet sources may be important.

Stable isotope analysis, a widely used tool for studying trophic ecology, can identify trophic transfer and the contribution of food sources to the diet of organisms if the potential food sources are isotopically distinct (Kling et al. 1992; Post 2002; Iverson et al. 2002; Parnell et al. 2008). Stable isotopes provide information on the assimilated diet integrated over the timescales of tissue growth and turnover, which themselves vary with tissue growth, temperature, food quality and quantity, respiration and excretion rates. Within any single food web, variations in stable isotopic (SI) composition of carbon (expressed as  $\delta^{13}\text{C}$  values) are frequently largely explained by isotopic differences in primary carbon sources at the base of the food web; while variations in nitrogen isotope ratios (expressed as  $\delta^{15}\text{N}$  values) predominantly (but by no means exclusively) vary with trophic position (Cabana and Rasmussen 1996; Peterson 1999). While stable isotope approaches have frequently been applied to study diet in medusae (McKenzie et al. 2014; Fleming et al. 2015; Javidpour et al. 2016), relatively little information regarding polyps is available.

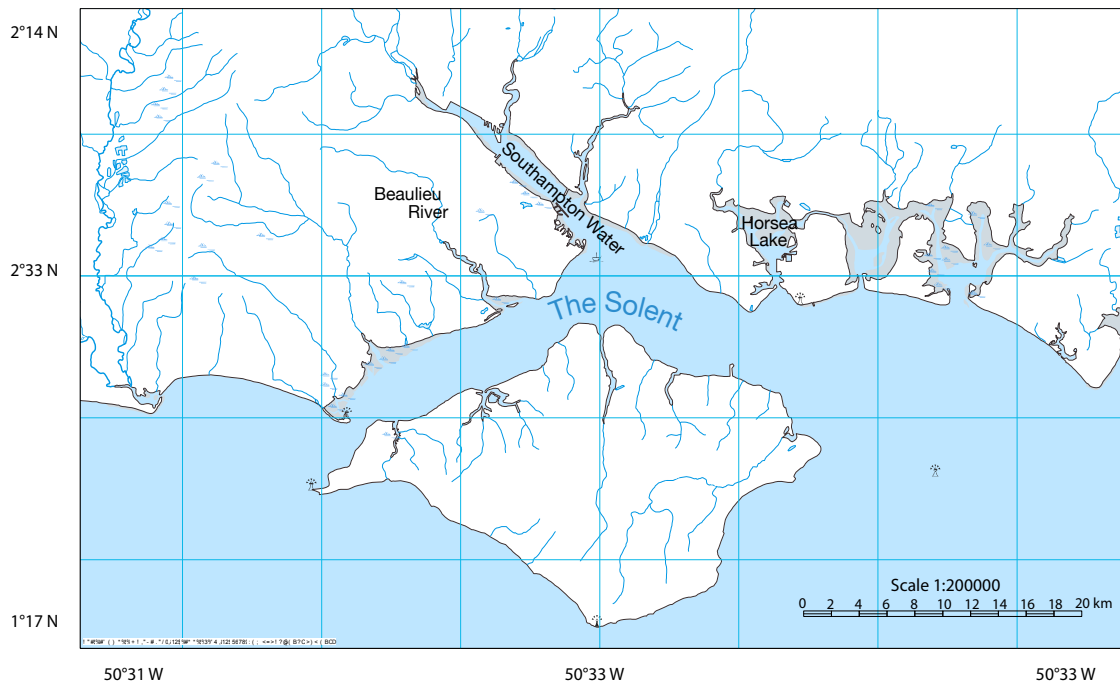
Here, dietary sources of *A. aurita* polyps in the field were investigated and compared between winter and summer conditions. Polyps were also raised under laboratory conditions commonly used to study jellyfish life cycles and stable isotope analyses employed to study the nutritional response of polyps to these laboratory conditions.

## 6.3 Materials and methods

### 6.3.1 Field location

Field experiments were conducted in the Beaulieu River (50° 80' 04.55" N / 1° 42' 28.12" W) (Fig. 6.1), an intertidal estuary that flows for 19 km south-westwards

through the New Forest into the Solent estuarine system and drains 80 km<sup>2</sup> of heathland and bog resulting in organic-rich water (Turner et al. 1998). The estuary is surrounded by saltmarshes and mudflats and has a tidal height of 4.4 m (Chen et al. 2011).



**Fig. 6.1** Study location of the Beaulieu River in southern Britain

### 6.3.2 Laboratory experiment

Fully-grown, healthy *Aurelia aurita* polyps of similar size were selected from the Beaulieu River stock culture, starved for 28 days, and placed into 6°C and 20°C incubators and maintained in well-aerated filtered seawater with salinity of 31. *Artemia* nauplii (<24 h old) were added daily. Water of the same temperature and salinity was replaced before each feeding event. Individual *A. aurita* polyps were detached carefully with tweezers from the settling plates, then washed and blotted dry on filter paper before being placed into pre-weighed tin-capsules (5 individuals per capsule) and frozen at -80°C. At the same time *Artemia* were collected and prepared as the polyps.

### 6.3.3 Field sampling

To quantify the extent that different nutrient sources support polyps in the field, terrestrial plants (e.g. *Spartina*, *Halimione*), wood litter (dried leaves, branches, bark), sediment from the saltmarsh, river sediment, zooplankton and phytoplankton were sampled weekly from January to May. Wood litter and plant materials were collected from the riverbank and samples were placed into 5 ml Eppendorf cups on ice before being frozen at -80°C. For riverine and saltmarsh sediment the top 1 cm of the sediment surface was collected, filled into 5 ml Eppendorf cups and transported on ice to the laboratory and frozen at -80°C. Zooplankton was collected with a 210 µm mesh plankton net, towed alongside a pontoon. Zooplankton samples were transferred into a 500 ml plankton bottle and transported back to the laboratory. Zooplankton (mainly crustacean copepods and decapods, polychaetes, barnacle larvae and mollusc larvae) were selected under a stereomicroscope with fine forceps, washed and placed into tin capsules before being frozen at -80°C. Zooplankton was not treated with acid prior stable isotope analysis as this method can change both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Schlacher and Connolly 2014). For the collection of phytoplankton, surface water was collected in 1 L plastic bottles. Seawater was filtered with a vacuum pump through a polycarbonate filter, and the remaining phytoplankton was scraped off the filter and placed immediately into pre-weighed tin capsules and frozen at -80°C. Five replicates were provided.

### 6.3.4 Field experiment

*Aurelia aurita* polyps obtained from a stock culture originating from the Beaulieu River population were placed out in the Beaulieu River at Buckler's Hard marina on settling plates (at about 1 m depth hanging from the pontoon) in two phases: during winter

(from 17 Feb 2016) and summer (from 20 Jul 2016). Sub-samples of these field-deployed polyps were collected weekly for six weeks, simultaneous with the environmental sampling. Samples of polyps were collected weekly for six weeks and prepared as above. Special care was taken to remove any debris from the polyp.

#### 6.3.5 Sample processing

Samples were freeze-dried (Thermo Scientific Heto PowerDry LL33000) at -50°C for 12 h (for plant 24 h) to a constant weight, and dry weights (DW) were measured using a microbalance (Sartorius ME5). Wood litter, plant and sediment samples were homogenised with a mortar and pestle, and weighed into pre-weighed tin capsules. The isotopic composition of carbon and nitrogen (expressed as  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  values) was determined at the East Kilbride Node of the Natural Environment Research Council Life Science Mass Spectrometry Facility via continuous flow isotope ratio mass spectrometry using an ECS 4010 elemental analyser (Costech, Italy) interfaced with a Delta XP mass spectrometer (Thermo Electron, Germany). The standard deviation of multiple analyses of an internal gelatine standard was about 0.17‰ for  $\delta^{13}\text{C}$  and 0.15‰  $\delta^{15}\text{N}$ . Stable-isotope concentrations were expressed as  $\delta$  notation as part per thousand (‰).

#### 6.3.6 Statistical analysis

Generalized additive models (GAMs) were used to investigate the relationship between the isotopic composition of polyps (response variable) and time and temperature (explanatory variables) during the experiments (as described in Zuur et al. 2010). Bayesian mixing models (SIAR, Parnell et al. 2008; Parnell et al. 2010) were used to estimate the proportional dietary composition of *in situ* polyps (SIAR, R package



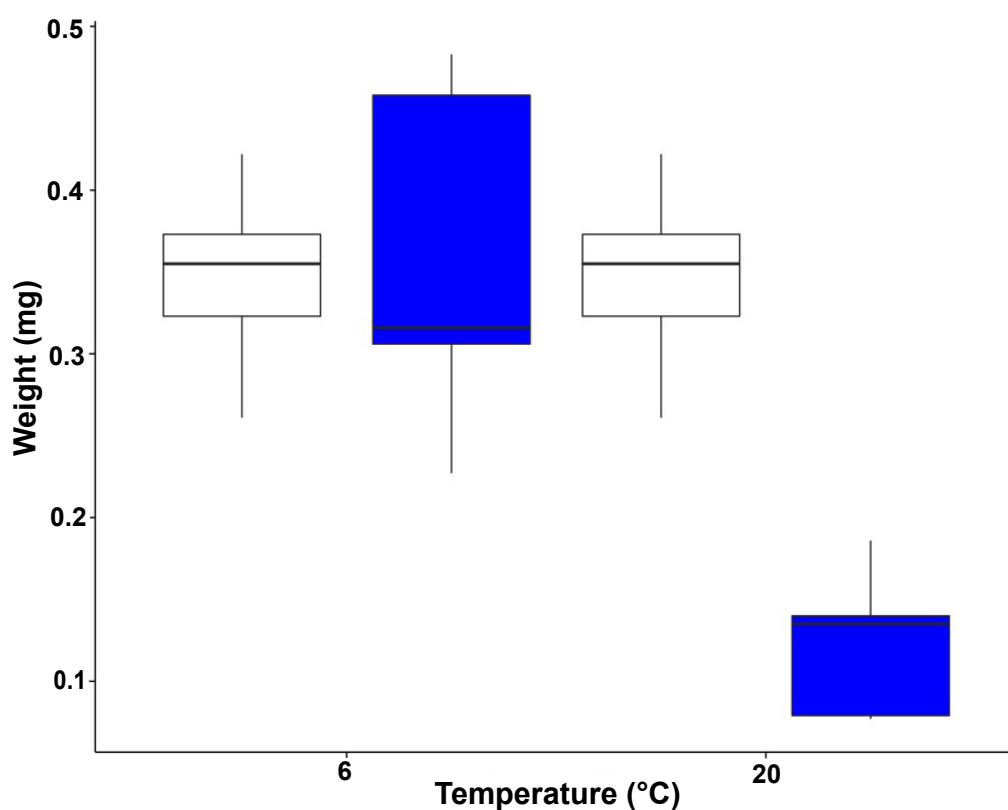
version 4.1.3, Parnell and Jackson (2011)). SIAR was run with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of *Aurelia aurita* after 42 days, to account for the turnover of the tissue (D'Ambra et al. 2014). Tissue-diet discrimination factors for polyps are unknown and here were estimated at 3 per mille for  $\delta^{15}\text{N}$  and 1 per mille for  $\delta^{13}\text{C}$  values. The aim of this analysis was to identify differences in diet sources between two populations of polyps (summer and winter), therefore while we assume that diet tissue isotopic fractionation terms are consistent between summer and winter, we do not draw any interpretations from estimated proportional contributions of potential diet sources (which would be sensitive to the absolute values of diet-tissue fractionation chosen).

Two separate models were run to explore the diet of *A. aurita* polyps, because environmental parameters vary between months (Fry and Quinones 1994). Plant, sediment and zooplankton were considered: in model A) for the diet of *A. aurita* polyps during winter, and in model B) for the diet of *A. aurita* polyps during summer. The dry weights of polyps at 6 and 20°C after 6 weeks were compared to the starting weight with a Two-way ANOVA.

## 6.4 Results

### 6.4.1 Laboratory experiment

The mean dry weight of *Aurelia aurita* polyps decreased by 41% after 6 weeks at 20°C (Two-way ANOVA:  $F_{1,16}=7.04$ ,  $p<0.05$ ). At 6°C the mean dry weight stayed similar, with 4% increase, over the experiment (Fig. 6.2).



**Fig. 6.2** *Aurelia aurita* polyp weights shown as boxplots at 20°C, 6°C (blue) and control (white). The dry weight at 20°C was 4% lower after 6 weeks (Two-way ANOVA:  $F_{1,16}=7.04$ ,  $p<0.05$ )



**Fig. 6.3** Daily mean surface water temperature (°C) at Buckler's Hard in the Beaulieu River, from 28th of May 2014 to 10th of February 2016. Temperature data were logged continuously, with discrete measurements taken every 5 minutes

As summer mean ( $\pm$  sd) water temperatures in the Beaulieu River (July to August) were  $20.04 \pm 1.91^\circ\text{C}$ , and during winter (January to February) were  $6.35 \pm 1.68^\circ\text{C}$  (Fig. 6.3) the summer temperature was chosen to be  $20^\circ\text{C}$  and the winter temperature was chosen to be  $6^\circ\text{C}$ . Stable isotope ratios are reported as described by Bond and Hobson (2012).

Isotopic values of nitrogen differed significantly between polyps maintained in the laboratory at 6 and  $20^\circ\text{C}$  with an increase of  $\delta^{15}\text{N}$  values by 1‰ in polyps at  $20^\circ\text{C}$  after 21 days (GAM:  $F=84.554$ ,  $p<0.0001$ , Table 6.1 and Fig. 6.4). At  $6^\circ\text{C}$ ,  $\delta^{15}\text{N}$  values in lab polyps decreased by 1.5‰ after the start but did not change significantly over the remaining time (42 days) (GAM:  $F=0.907$ ,  $p>0.05$ ).  $\delta^{13}\text{C}$  values decreased by 1.5‰ over time (GAM:  $F=44.7983$ ,  $p<0.0001$ ), but  $\delta^{13}\text{C}$  values did not differ in polyps maintained at 6 and  $20^\circ\text{C}$  (GAM:  $F=3.4667$ ,  $p>0.05$ ) (Table 6.2 and Fig. 6.4). The mean ( $\pm$  sd) isotopic composition of *Artemia* was  $13.79 \pm 0.86\text{‰}$  for  $\delta^{15}\text{N}$  values and  $-21.71 \pm 0.30\text{‰}$  for  $\delta^{13}\text{C}$  values ( $n=13$ ).

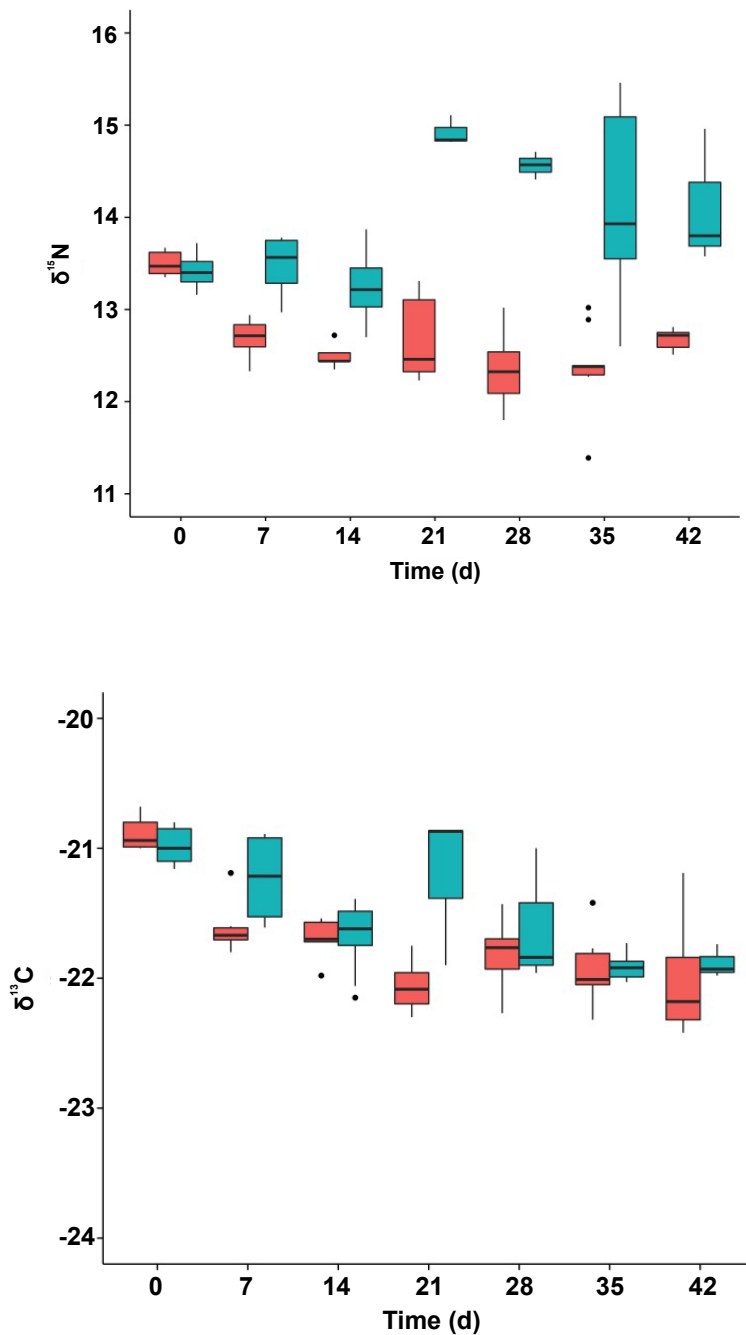
**Table 6.1** Results of the generalized additive model (GAM). The effect of temperature and the interaction between time and temperature were significant on the  $\delta^{15}\text{N}$  isotopic composition of laboratory polyps

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Time	1	0.2715	0.2715	0.907	0.3439
Temperature	1	25.3073	25.3073	84.554	5.043e-14 ***
Time:Temperature	1	6.4812	6.481	21.654	1.334e-05 ***
Residuals	77	23.0464	0.2993		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

**Table 6.2** Results of the generalized additive model (GAM). The effect of time was significant on the  $\delta^{13}\text{C}$  isotopic composition of laboratory polyps

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Time	1	4.8698	4.8698	44.7983	3.004e-09***
Temperature	1	0.3769	0.3769	3.4667	0.06638
Residuals	78	8.4790	0.1087		

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

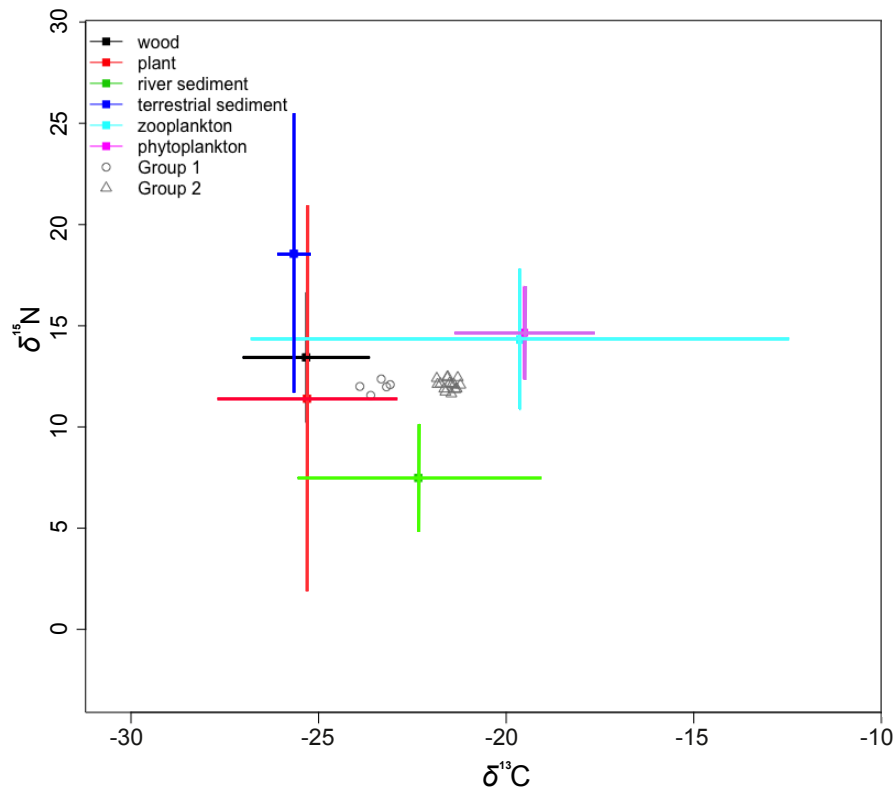


**Fig. 6.4** *Aurelia aurita* polyp  $\delta^{15}\text{N}$  (top) and  $\delta^{13}\text{C}$  (bottom) isotopic composition at 6°C (red) and 20°C (green) over 42 days in the laboratory

#### 6.4.2 Field experiment

In the Beaulieu River the water temperatures ranged from 3 to 24°C (Fig. 6.3).

The mean  $\pm$  sd isotopic compositions ( $\delta^{15}\text{N}$  /  $\delta^{13}\text{C}$  values) of carbon and nitrogen in potential food sources from the Beaulieu River ranged from aquatic sources including phytoplankton ( $11.65 \pm 0.53$  /  $-23.53 \pm 0.89\text{‰}$ ) and zooplankton ( $11.32 \pm 1.41$  /  $-23.63 \pm 3.57\text{‰}$ ), to terrestrial sources including sediment ( $15.56 \pm 3.27$  /  $-29.66 \pm 0.05\text{‰}$ ), wood litter ( $10.43 \pm 1.24$  /  $-29.33 \pm 0.82\text{‰}$ ), plant ( $8.39 \pm 4.64$  /  $-29.31 \pm 1.17\text{‰}$ ) and river sediment ( $4.48 \pm 0.84$  /  $-26.54 \pm 1.60\text{‰}$ ) (Fig. 6.5). Terrestrial plant sources were depleted in  $\delta^{13}\text{C}$  values relative to phytoplankton, reflecting exclusively C-3 vegetation sources. Sediment from the river showed depleted  $\delta^{15}\text{N}$  values and  $\delta^{13}\text{C}$  values midway between aquatic and terrestrial plants.



**Fig. 6.5** Biplot of the stable isotopic composition of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of potential food sources including plant, wood litter, sediment from the river and saltmarsh, phytoplankton and zooplankton. Consumers (polyps) during winter (Group 1) and during summer (Group 2)

### 6.4.3 Thermal variability in field polyps stable isotope values

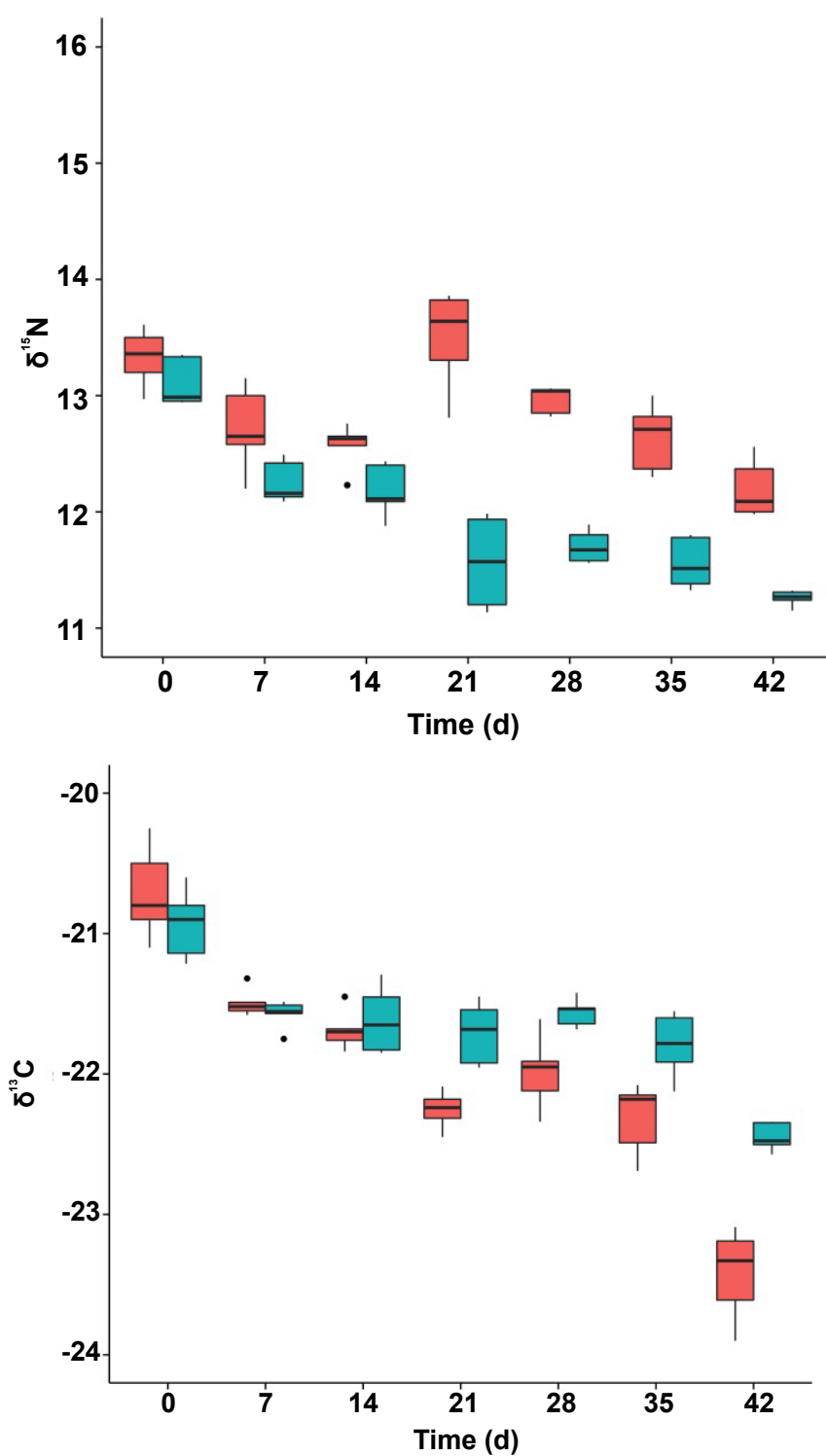
$\delta^{15}\text{N}$  values of field-deployed experimental polyps changed significantly over time (GAM:  $F=26.173$ ,  $p<0.0001$ ) (Table 6.3 and Fig. 6.6). While the  $\delta^{15}\text{N}$  values of field-deployed polyps decreased in summer by 2‰ over 42 days, in winter the  $\delta^{15}\text{N}$  first decreased by about 1‰ then increased sharply after 21 days by 1.5‰ before decreasing again. Overall,  $\delta^{15}\text{N}$  values declined over time in both field experiments and were significantly lower in summer compared to winter (GAM:  $F=85.923$ ,  $p<0.0001$ ).  $\delta^{13}\text{C}$  values of field polyps decreased over 42 days (GAM:  $F=185.645$ ,  $p<0.0001$ ), with a greater decrease in winter compared to summer (GAM:  $F=15.358$ ,  $p<0.0001$ ) (Table 6.4, Fig. 6.5 and 6.6).

**Table 6.3** Results of the generalized additive model (GAM). The effect of time, temperature and the interaction between time and temperature were significant on the isotopic composition of  $\delta^{15}\text{N}$  of field polyps

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Time	1	10.0018	10.0018	26.173	4.672e-11***
Temperature	1	13.8225	13.8225	85.923	1.665e-13***
Time:Temperature	1	1.6539	1.6539	10.281	0.002086 ***
Residuals	65	10.4566	0.1609		
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					

**Table 6.4** Results of the generalized additive model (GAM). The effect of time, temperature and the interaction between time and temperature were significant on the isotopic composition of  $\delta^{13}\text{C}$  of field polyps

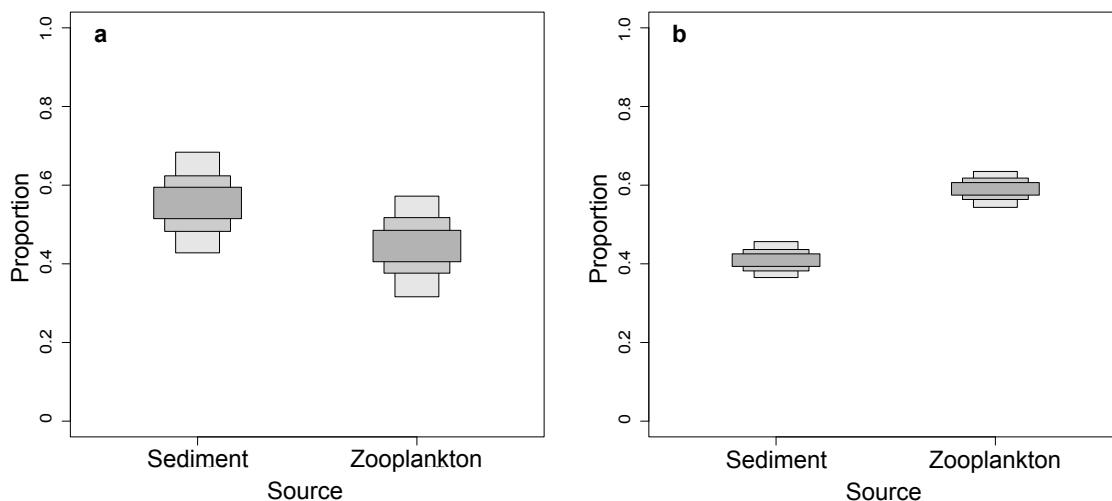
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Time	1	20.1849	20.1849	185.645	<2.2e-16***
Temperature	1	1.6698	1.6698	15.358	0.0002164***
Time:Temperature	1	2.3703	2.3703	21.800	1.564e-05***
Residuals	65	7.0674	0.1087		
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					



**Fig. 6.6** Isotopic composition of  $\delta^{15}\text{N}$  (top) and  $\delta^{13}\text{C}$  (bottom) of *Aurelia aurita* polyps in the field during winter (red) and summer (green) over 42 days in the field

#### 6.4.4 Contribution of prey sources to the diets of *Aurelia aurita* polyps

The SIAR mixing model was used to quantify differences in proportional diet sources supporting *Aurelia aurita* polyps between winter and summer experiments. Estimated source contributions suggested a shift from nutrient sources dominated by terrestrial plant debris in winter to a more mixed source contribution in summer (Fig. 6.7). In both cases, zooplankton did not account for a major contribution towards the diet of *A. aurita* polyps.



**Fig. 6.7** Proportion (%) of benthic and pelagic food sources of *Aurelia aurita* polyp during **a** winter and **b** summer calculated using SIAR. Dark grey of the box plots indicates 95%, lighter grey indicates 75% and the lightest grey indicates 25% confidence intervals. See supplementary material Table A4.1 and Fig. A4.1

## 6.5 Discussion

### 6.5.1 Laboratory experiment

The isotopic composition of *Aurelia aurita* polyps during the laboratory experiment reveals differences in the nutritional suitability of *Artemia* between the two temperature conditions. Because of their small size and lack of motility polyps likely have lower energy demands for basic metabolism and growth compared to medusae (Kamiyama



2011). At 6°C growth of polyps as indicated by weight changes over the 42 days was limited, presumably because of their low metabolic rate. Metabolic rate indirectly measured as respiration rate in polyps have been observed to be lower at colder temperatures (e.g. 7°C) when compared to warmer temperatures (e.g. 15°C) (Mangum et al. 1972; Gambill and Peck 2014; Höhn et al. 2017). Accordingly, *A. aurita* polyps showed only limited isotopic shift towards the composition of *Artemia*.

At 20°C, polyps showed a marked reduction in body mass, indicating that food sources provided were either indigestible, or insufficient to meet the metabolic requirements of the higher temperature. Furthermore, close observations of polyps during the experiment revealed that they looked unhealthy (i.e. reduced size, “softer” texture, paler colouration, shorter tentacles). Weight loss coincided with increasing  $\delta^{15}\text{N}$  values, consistent with catabolism of body reserves to provide basal metabolic requirements (e.g. starvation). Similarly, laboratory experiments by Kamiyama (2013) using ciliates as food for *A. aurita* polyps suggest that ciliates are more easily ingested and absorbed by the polyp than *Artemia*. Respiration rates of *Aurelia* polyps from the Baltic Sea and from Scotland have been observed to decrease at 20°C, indicating that polyps might have reached their thermal limit at that warm summer temperature (Gambill and Peck 2014).

#### 6.5.2 Field experiment

The significant shift in the isotopic values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in *Aurelia aurita* polyps maintained on settling plates in the field over a period of 6 weeks demonstrates assimilation of new food (i.e. successful feeding) during the course of the experiment. During both the winter and summer season, polyps'  $\delta^{15}\text{N}$  values declined from the starting laboratory *Artemia*-raised signal, indicating ingestion of new food sources with

relatively low  $\delta^{15}\text{N}$  values. In the Beaulieu River, *in situ* phytoplankton, zooplankton and saltmarsh sediment sources were all relatively enriched in  $\delta^{15}\text{N}$  values comparative to *Artemia*, and in-river sediment was the only sampled nutrient source that was depleted in  $\delta^{15}\text{N}$  when compared to *Artemia*. The consistent decrease in  $\delta^{15}\text{N}$  values following deployment in the field therefore indicates that polyp metabolism was predominately supported from benthic pathways such as sediment-dwelling or epifaunal bacteria, epibenthic organisms and detritivores. The carbon assimilated by polyps was markedly more negative in winter than in summer, suggesting that in winter the organisms comprising polyp diet were largely supported by terrestrial plant debris whereas in summer polyp food is more tightly linked to pelagic production, most likely via bacterial or detritivore reworking of sedimented phytoplankton. The total zooplankton abundance varies in the Beaulieu River through the season, with low zooplankton abundance of 119 ind.  $\text{m}^{-3}$  in April (2014) increasing to a high of 821 ind.  $\text{m}^{-3}$  in July, indicating that the zooplankton abundance is highly seasonal and decreases during the cold winter months (Höhn unpubl data). While *Aurelia aurita* medusae primarily predate on mixed mesozooplankton (Gröndahl 1988; Östman 1997), this was not observed to be polyps' main diet during the course of the experiment. The size differences might be too small between zooplankton and polyps and the swimming speed too fast, decreasing the chance of capturing zooplankton (Östman 1997; Kamiyama 2013). Instead *A. aurita* polyps have been observed to feed on microzooplankton (e.g. planktonic ciliates) in laboratory experiments (Kamiyama 2011).

In this study we have demonstrated that the previous-held assumption that *Aurelia* growth and metabolism of the polyp life stage of *Aurelia* is only supported by pelagic

sources such as zooplankton, is in fact incorrect, at least in shallow tidal estuaries. In the Beaulieu River polyps' diet changed from a phytoplankton or sediment-derived source in summer towards a benthic, terrestrial-plant derived source in winter when pelagic plankton productivity is at its lowest. We showed that in winter, ingested carbon was incorporated into somatic growth, resulting in bigger polyps, which can then transform into strobilae consisting of a greater number of discs, thus producing more ephyrae per polyp (Wang et al. 2015). Laboratory polyps maintained on *Artemia* failed to meet their metabolic costs, especially at 20 since they were not extracting sufficient nutrients. As a result, reproductive rates (strobilation) of *Artemia*-fed polyps (standard procedure see Purcell et al. 2013) might not reflect natural rates. Predictions of cascading effects such as bloom events based on laboratory-based estimates of reproductive metabolism should be approached with caution.

## 6.6 Conclusions

This study provides evidence of a difference in *Artemia* assimilation by *Aurelia aurita* polyps at two different temperatures. While at 6°C the metabolic rate and thus assimilation of *Artemia* was restricted, at 20°C polyp's metabolic cost exceeded the assimilation of *Artemia* leading to starvation. Stable isotope analyses highlighted a contribution of benthic-pathway food sources in the diet composition of *A. aurita* polyps in the Beaulieu River and limited ingestion of zooplankton as previously believed. This study implies that laboratory-based observations of physiological rates or ecological behaviours in polyps that are nourished by *Artemia* may not be representative of wild populations. Finally, if *A. aurita* polyps metabolic rate decreases at temperatures of 20°C, expected warming ocean temperatures caused by climate change, would likely have a negative effect on their survival and reproduction.



# Chapter 7

## Synthesis & Conclusions

## 7. Synthesis & Conclusions

### 7.1 Background, Aims and Objectives

Gelatinous zooplankton make a major contribution (38.3 Tg C; when assuming a global ocean area of 361,900,000 km<sup>2</sup>) in the mixed layer of the ocean (Lucas et al. 2014).

Jellyfish blooms have been observed to increase in several regions of the world (Brotz et al. 2012) and recent studies indicate that anthropogenic influences including ocean warming, over-fishing, eutrophication and coastal structures could be the causes of increased gelatinous zooplankton populations (Lynam et al. 2006; Doyle et al. 2007; Richardson et al. 2009; Condon et al. 2011). Therefore, jellyfish research has gained increasing attention (Haddock 2004) but there are still knowledge gaps in the complex life cycle of scyphozoans (Berrill 1952; Gröndahl 1988; Arai 1997; Lucas 2001). While research in neurobiology, physiology and development ecology in the pelagic medusa life stage has increased, laboratory experiments on the sessile polyp life stage are still rather rare (Gambill and Peck 2014; Pascual et al. 2014; Schiariti et al. 2014). The common bloom-forming scyphozoan *Aurelia aurita* has a metagenic life cycle including a sexually-reproducing medusa and an asexually-reproducing benthic polyp (Spangenberg 1967). The effect of environmental factors influencing the survival, growth and asexual reproduction of the perennial benthic life stage may directly affect the distribution and abundance of future medusa populations. Thus, both *A. aurita* life stages (medusa and polyp) were studied in their natural environment and in the laboratory in this thesis to understand bloom formation in a widely distributed species in northwest Europe.

The aims of this thesis were to study population variability of *Aurelia aurita*, with a close focus on the polyp life stage. A natural local medusa population was sampled and

monitored along with environmental parameters, to determine how the species is adapted to its environment in terms of growth, longevity and reproduction. *A. aurita* polyps from different locations in northwest Europe were sampled and maintained under controlled conditions. I designed and conducted experiments on polyps, to gain a better understanding on polyp's physiological response to temperature, such as reproduction and metabolism. Polyp populations in their natural environment were monitored over 12 month, to observe population growth and recruitment of new medusae (strobilation). Furthermore, the metabolism of polyps maintained on *Artemia*-diet was compared with natural polyps during summer and winter (conditions). Still, there is little information on the metabolism of *A. aurita* polyps and on how environmental factor affect fundamental metabolic processes. Information about metabolic processes is necessary for the prediction of future bloom events, in particular with increasing water temperatures due to global warming.

**The objectives of the thesis were to:**

- 1) Describe the annual life cycle of growth and development of a natural local *Aurelia aurita* population, and identify population variability in growth and reproduction along well-studied closely located populations.
- 2) Measure respiration rates of *Aurelia aurita* polyps as a function of temperature exceeding their natural temperature window, to identify thermal limits and observe population specific thermal tolerances of populations from different locations.

- 3) Examine the effect of temperature on asexual reproduction of *Aurelia aurita* polyps from different locations to investigate population specific acclimation processes.
- 4) Describe the annual population dynamics of *Aurelia aurita* polyps in a natural environment and identify *in situ* abundance and strobilation activity. Strobilation rates were compared with laboratory-derived observations.
- 5) Observe natural food sources for wild polyps and compare *Artemia*-fed polyps in the laboratory with wild polyps. Compare the quality between natural food sources and *Artemia* to identify natural metabolic rates of *Aurelia aurita* polyps.

The main findings that are relevant to these objectives are addresses within the scientific chapters 2 to 6. Here, I provide a synthesis of these findings and draw general conclusions regarding their implications.

## **7.2 Examining population variability of *Aurelia aurita* medusae**

*Aurelia aurita* populations have been studied from a number of locations in northern Europe, indicating variability in response to local environmental conditions. In southern England, two distinct *A. aurita* populations in Southampton Water and in Horsea Lake have been studied (Lucas and Williams 1994; Lucas 1996). For the first time, *A. aurita* medusa populations in the closely located Beaulieu River have been investigated and compared to Southampton Water and Horsea Lake populations (Chapter 2). Medusae have been observed to grow and mature analogous to other temperate populations with a



short appearance of the pelagic life stage (3 to 6 months) during the warm spring and summer months. Interannual differences in the timing, occurrence and sizes of medusae were observed to coincide with the physical and biological conditions of the Beaulieu River. Short longevity, large medusa sizes of small abundance and high mesozooplankton levels were observed in 2014. In contrast, prolonged longevity, small medusae sizes of high abundance and low mesozooplankton levels were recorded in 2015. These, observed density dependent population dynamics are in agreement with results published by Schneider and Behrends (1998) from the Kiel Bight, Germany. Monitoring medusa population variability in accordance with environmental variables is of importance, because mechanisms that lead to bloom events can be identified. According to Chapter 2, *A. aurita* populations from closely-located ecosystems differ, which may indicate physiological adaptation to the physical and biological parameters of their specific environment. These results agree well with other *A. aurita* population studies in northwest Europe (Chapter 2 Table 2.1; Lucas 2001). Expected increasing sea temperatures arising from global climate change may affect population size as growth was positively correlated to temperature. However, there is still little known about the benthic life stage (polyp) and its response to temperature, which might be the key to the recruitment success of new medusa. Consequently, the ecology and physiology of the polyp life stage was focus of the remaining four thesis chapters.

### **7.3 The effect of temperature on the respiration rates of three *Aurelia aurita* polyp populations**

The perennial polyp life stage of *Aurelia aurita* contributes to the seasonal occurrence and abundance of medusa blooms by asexual reproduction. Here the physiology of the small largely unknown polyp life stage has been investigated (Chapter 3). So far, only

one study has measured respiration rates of *A. aurita* polyps (from northern Europe) versus a number of temperatures (Gambill and Peck 2014). In this study the respiration rates of *A. aurita* polyps in response to temperature exceeding their natural range (from 2 to 22°C) from three northwest European locations, including southern England, Scotland and Norway were measured to examine polyp's thermal tolerance window. Thermal tolerance windows of jellyfish polyps are of particular interest, as they will help to predict the recruitment of new medusa and facilitate the inclusion of jellyfish and the sessile life stage in ecosystem models.

Respiration rates were significantly different between the three polyp populations, indicating acclimation to their different natural thermal tolerance window. A critical temperature was reached at 14°C, with lower respiration rates above and below that temperature. A similar critical temperature was observed in respiration rates of *A. aurita* polyps from the Baltic Sea and NW Atlantic (Gambill and Peck 2014). Whilst polyps from Norway showed a less pronounced pattern, surprisingly, they survived (at least temporally) temperatures exceeding their natural range (see Chapter 4). This investigation showed that differences in polyp's respiration rates across their distributional range in northwest Europe exist, indicating that populations have evolved adaptations to local environmental thermal conditions (Chapter 3 and 4). Findings of this study agree well with geographic differences found in survival and asexual reproduction of *Aurelia* polyps from the Mediterranean Sea, Baltic Sea and Red Sea by Pascual et al. (2014). The respiration rates of the Scottish and the southern England populations declined after 14°C indicating that polyp may not be as tolerant to global warming as originally thought. Therefore, increasing temperatures due to global greenhouse gas emission may limit survival and reproduction of polyps with a knock-on effect on medusa populations.

#### **7.4 Effect of temperature on asexual reproduction of *Aurelia aurita* polyps, in three different populations: Beaulieu River, Horsea Lake and Fiskebøl**

While the effect of temperature on respiration rates of *Aurelia aurita* polyps from three different locations was studied in the previous Chapter (Chapter 3), here experiments on the effect of temperature on asexual reproduction of different populations were carried out (Chapter 4). Temperature plays a key role in the onset and extent of asexual reproduction of *A. aurita* polyps, because polyps reproduce at different times of the year and in multiple ways. Previous studies suggest greater reproductive output of polyps in warmer temperatures, leading to increasing numbers of jellyfish with global warming. To measure the reproductive success of *A. aurita* populations, polyps from three different environments were maintained at three temperatures of 12, 16 and 18°C for 36 days. Their reproductive rates were monitored and most buds were produced by Horsea Lake polyps at 16°C, followed by Beaulieu River polyps that produced maximum buds at 12°C. Similarly, the Norwegian population had highest budding and survival rates at 12°C in comparison to the higher temperatures. In agreement with these findings, Purcell et al. (2012) observed a decrease in asexual reproduction of polyps at higher temperatures. However, increasing budding rates with temperature were reported for *A. aurita* polyps from Helgoland and the Baltic Sea (Pascual et al. 2014; Schiariti et al. 2014). The mass (weight) of the southern England population decreased with increasing temperatures, indicating that metabolic demands could not be met at warmer temperatures. These findings agree with Han and Uye (2010) who found larger calyx diameter of *A. aurita* polyps at the lowest tested temperature. Only a few podocysts were produced during the experiment, and there were no clear differences between the three populations or temperature ranges. Strobilation was not observed during the experiment, indicating that polyps need a temperature change from high to low to

induce strobilation. Similar to the previous chapter (Chapter 3), the Norwegian population showed the ability to adapt to higher temperatures as polyps were able to survive and reproduce at temperatures up to 18°C. Overall, there was a difference in reproduction between the three populations, indicating adaptation of *A. aurita* polyps to their natural environment (Chapter 2, 3 and 4). However, higher temperatures expected by global warming might be harmful for polyps.

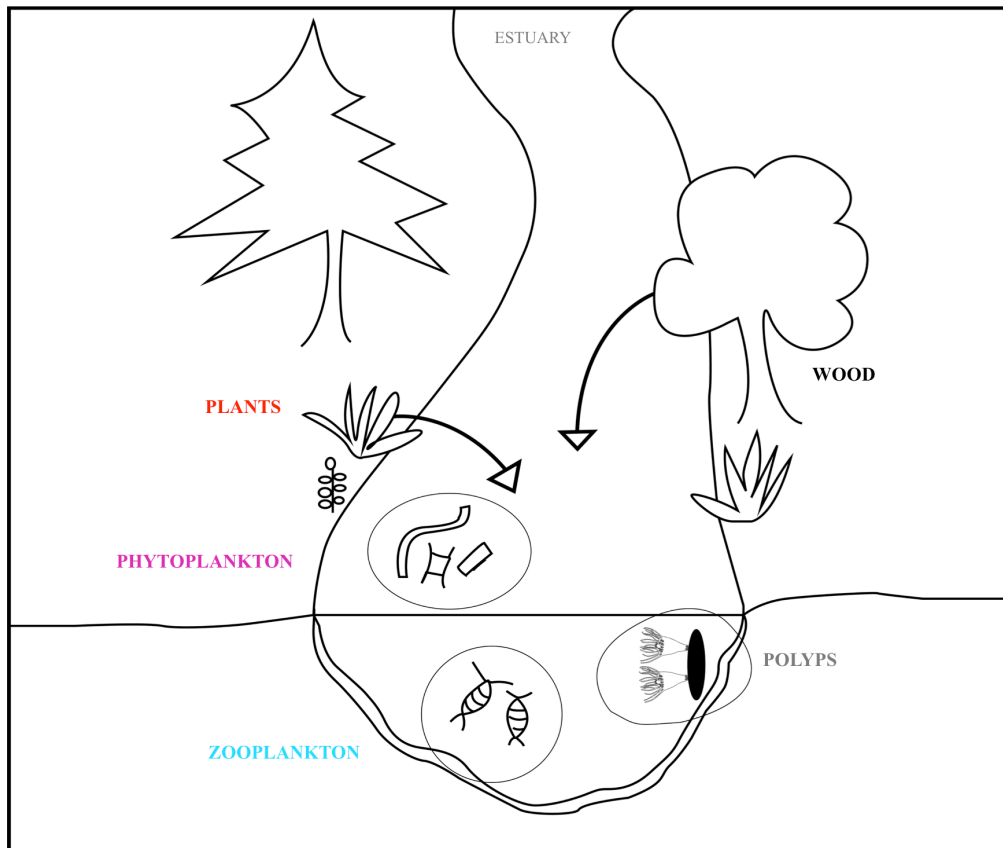
### **7.5 Examining polyp population dynamics and strobilation in a natural environment**

The effect of temperature on asexual reproduction of *Aurelia aurita* polyps from different locations was investigated in the laboratory within Chapter 3 and 4. In the next Chapter (Chapter 5) *in situ* population densities were correlated to natural temperatures. Natural strobilation cycles of bloom forming jellyfish polyps, which are thought to be one of the key drivers in the potential formation of jellyfish bloom events, have rarely been studied in *in situ* populations. A photographic survey of polyps of the common jellyfish *Aurelia aurita* in their natural environment in Horsea Lake was conducted for 12 month. Image analysis used to estimate polyps density over time, indicated that polyp density decreased from the summer to the winter month. Budding was only observed during July to October. Population growth including budding might depend on increasing temperatures and food availability typically for the summer month (Purcell et al. 1999; Wang et al. 2014). Strobilation commenced in December and continued until April (winter month) with peak incidence of polyp strobilation (50% of population) in January when water temperatures were at their minimum. Strobilation was correlated with temperature as the amount of strobilating polyps decreasing with increasing temperatures. Decreasing water temperatures have been proven many times to induce

strobilation (Kakinuma 1975; Miyake et al. 2002; Ishii et al. 2008; Holst 2012; Purcell et al. 2012). The duration of strobilation was prolonged in Horsea Lake, with ephyrae production spanning from December to April confirming past observations of ephyrae in the water column (Lucas 1996). A lower number of discs per strobila in comparison to laboratory polyps (i.e. 4 compared with 7) suggest a food-limited environment, as published experiments have shown that higher food availability increases ephyra production per polyp (Thiel 1962; Wang et al. 2014). I conclude that there is a highly seasonal but prolonged strobilation period in Horsea Lake and that laboratory strobilation experiments on *Artemia*-fed polyps might not reflect natural strobilation rates. Therefore, natural feeding rates of *in situ* polyps were studied in the next Chapter (Chapter 6).

#### **7.6 Insights into the feeding and bioenergetics of jellyfish polyps in wild and laboratory conditions: do experiments overestimate natural functional rates?**

The natural feeding ecology of small scyphozoan polyps, many of which are thought to be drivers of jellyfish blooms, has not been studied in *in situ* populations. The bioenergetics of *in situ* *Aurelia aurita* polyps in the Beaulieu River during summer and winter was studied and compared to laboratory polyps maintained on *Artemia* at 6 and 20°C (Chapter 6). It has been hypothesised that polyps feed of zooplankton similar to the medusa life stage (Kamiyama 2011, 2013). The stable isotopic composition of polyps and potential prey was measured to estimate the proportion of potential dietary sources (Fig. 7.1) to the diet of *A. aurita* polyps. However, mixing models showed that polyps were predominantly supported by benthic pathways and not by pelagic pathways. The primary carbon source shifted from a terrestrial plant-derived source in winter towards a phytoplankton or sediment-derived source in summer.



**Fig. 7.1** Stable isotopes were measured in all sources of organic material in the Beaulieu River

Unlike the medusa life stage, the polyp has to persist through the cold winter to recruit the next medusa generation. Polyps maintained in the laboratory assimilated *Artemia* food at 6°C although metabolic processes were reduced, but at 20°C polyps starved as their increased metabolic costs could not be supported from the *Artemia* diet. These ecological insights suggest that *A. aurita* polyps in the Beaulieu River are not preliminary supported by zooplankton prey and switch their diet from a terrestrial and sediment derived winter diet to a sediment and plankton dominated summer diet. This study indicated that experiments on growth and asexual reproduction of *Artemia*-fed polyps (Chapter 3 and 4) might not reflect natural rates of medusa recruitment especially at warmer temperatures (20°C), because resources extracted from *Artemia* are not sufficient to fuel metabolic costs (Chapter 3). The implications of this study

were to simulate field conditions of the metabolic rate of polyps in the laboratory.

Decreasing metabolic rates at temperatures of 20°C indicate that expected warming ocean temperatures caused by climate change, would likely have a negative effect on polyps survival and reproduction.

## 7.7 Final conclusions

This thesis showed that differences exist in the life cycle, growth and reproduction of closely located *Aurelia aurita* medusa populations from southern England. These differences are mainly caused by the physical and biological parameters of the environment (Chapter 2). Temperature has been shown to be a critical factor influencing the respiration rates of *A. aurita* polyps (Chapter 3) and their reproductive rates (Chapter 4 and 5). Temperature is known to be an important environmental factor for marine organisms, because all biochemical reactions are controlled by temperature. However, thermal sensitivity alone was not sufficient to explain the metabolic rate of *A. aurita* polyps. Food has a significant effect on the physiology of organisms, as organisms need energy to maintain body processes including growth and reproduction. Thus, in Chapter 6 the ingestion of lab and field based polyps and the nutritional value of food sources was investigated. Stable isotope analysis and mixing models revealed that *Artemia* is not a sufficient diet for polyps. Furthermore, experiments on the reproduction of *Artemia* maintained polyps might not reflect natural rates - possibly overestimating medusa recruitment (Chapter 5 and 6). Finally, *Aurelia aurita* in northwest Europe are probably not as tolerant to warming ocean temperatures as originally thought (Chapter 3 to 6), owing to observed thermal sensitivity at temperatures above 14°C. Nevertheless, polyps from Norway were able to adapt (temporarily) to temperatures above their natural thermal range, indicating flexibility in

the cold water population acclimating to warmer temperatures (Chapter 3 and 4). This thesis highlight the importance of including the sessile polyp life stage as well as local environmental conditions into models on the prediction of *A. aurita* populations.



# Appendices

A1 Appendix for Chapter 3

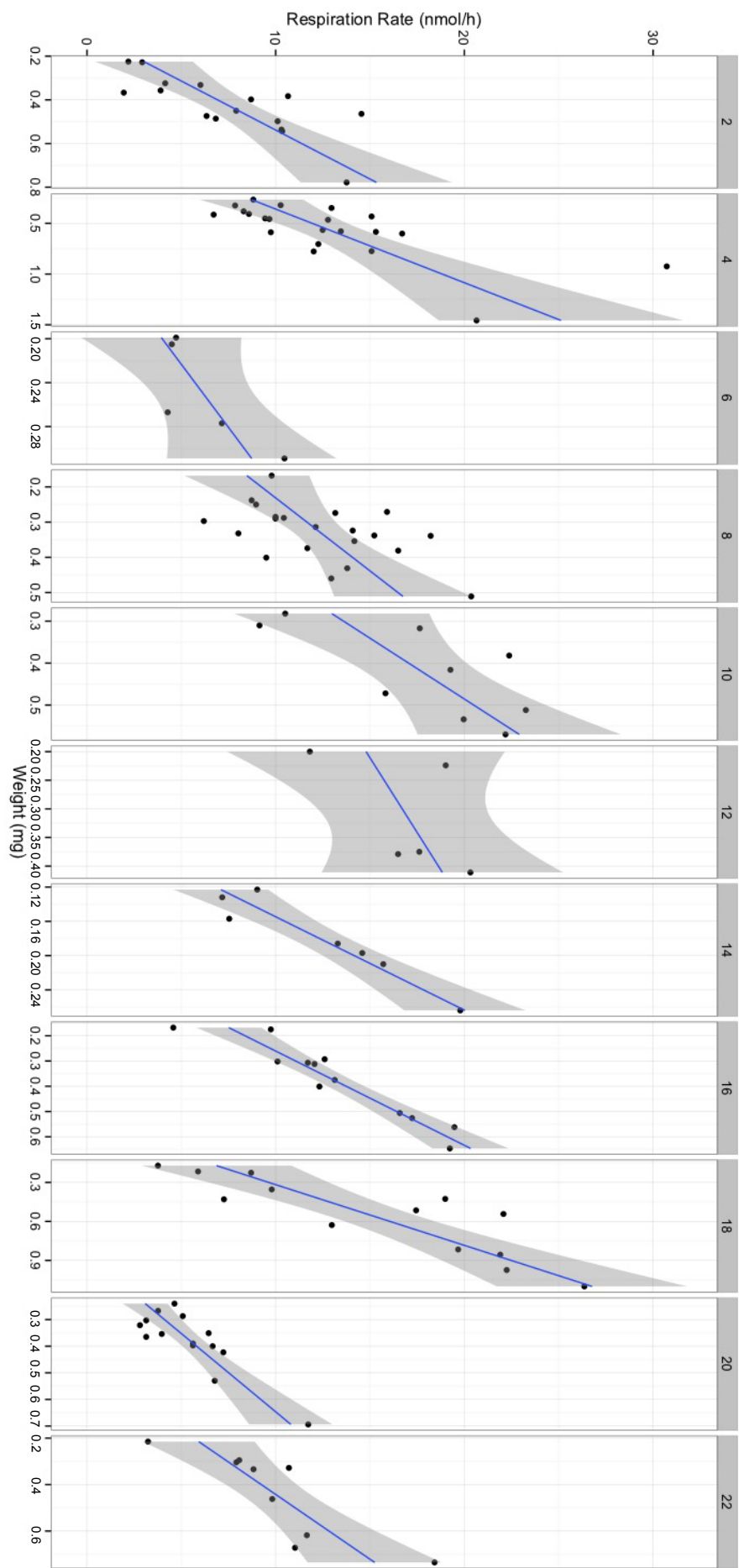
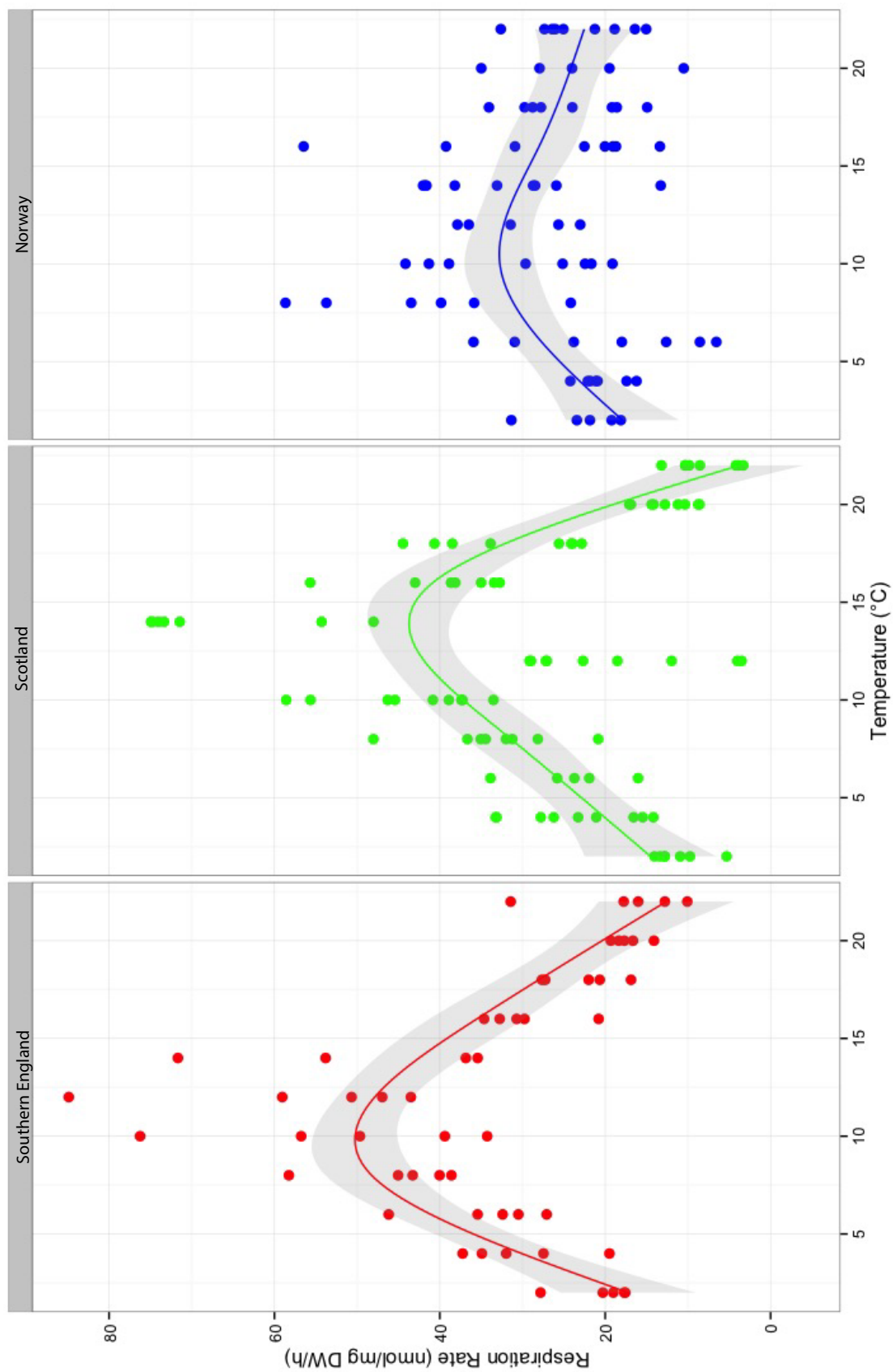


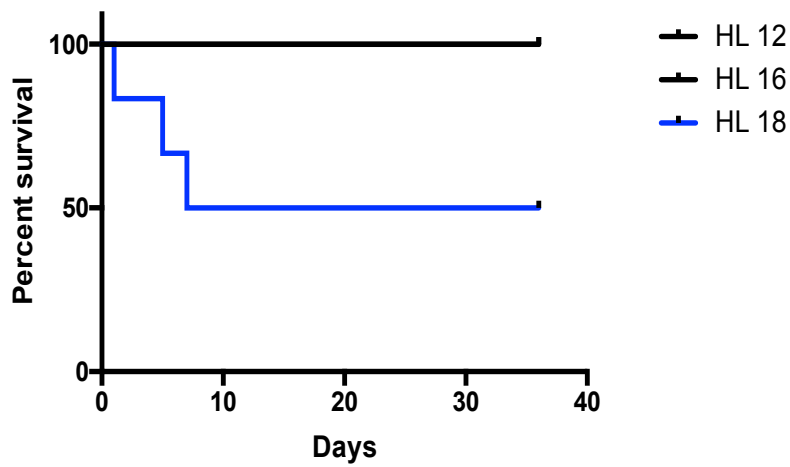
Fig. A1.1 Respiration rates ( $\text{nmol h}^{-1}$ ) versus weight (mg) of *Aurelia aurita* polyps expressed as a linear regression line



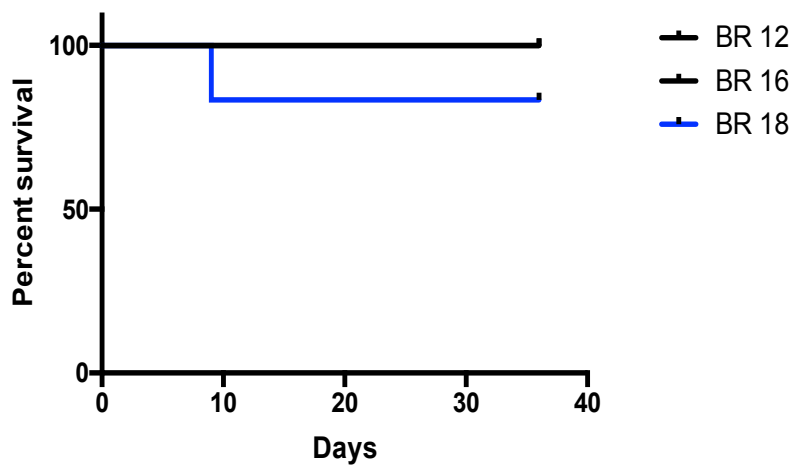
**Fig. A1.2** Respiration rates (nmol O<sub>2</sub> mg DW<sup>-1</sup> h<sup>-1</sup>) versus temperatures. Three populations are compared: southern England, Scotland and Norway. Scatter plot plus smoothing curve

## A2 Appendix for Chapter 4

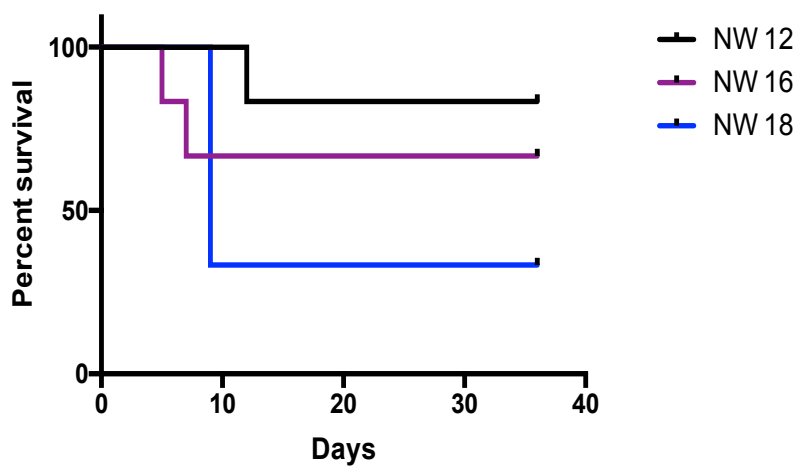
### Survival proportions: Survival of Horsea Lake



### Survival proportions: Survival of Beaulieu River



### Survival proportions: Survival of Norway



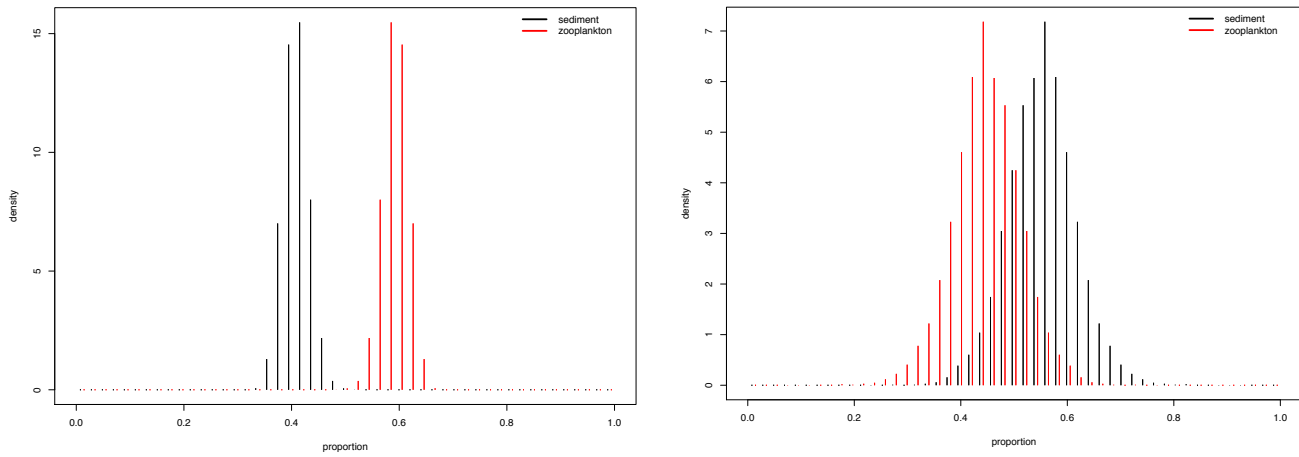
**Fig. A2.1** The survival in proportion (percentage survival) of all three populations (Beaulieu River, Horsea Lake, Norway) at the three temperatures (12°C, 16°C, 18°C) over time. All populations showed lowest survival at 18°C

A3 Appendix for Chapter 5



A3.1 Photographs of the sampling site and *Aurelia aurita* in Horsea Lake

## A4 Appendix for Chapter 6



**Fig. A4.1** Proportion (%) of *Aurelia aurita* polyp food sources during **a** winter and **b** summer calculated using SIAR

**Table A4.2** SIAR input

	sample type:	mean $\delta^{13}\text{C}$ ( $\pm$ sd)	mean $\delta^{15}\text{N}$ ( $\pm$ sd)
<b>Sources</b>	sediment	4.48 (0.84)	26.39 (1.60)
	plankton	12.42 (1.41)	19.82 (0.27)
<b>Consumers</b>	polyps summer	12.09 (0.26)	21.52 (0.19)
	polyps winter	11.98 (0.30)	23.42 (0.33)

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