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Contribution to the Theme Section 'Jellyfish bloom research: advances and challenges'

Winter river discharge may affect summer estuarine jellyfish blooms

Katherine Amorim[1], Ramona M. Mattmüller[2], María Algueró-Muñiz[3], Cédric L. Meunier[3], Santiago Alvarez-Fernandez[3], Maarten Boersma[2,3], Pedro Morais[1,4], Maria A. Teodósio[1,*]

[1]CCMAR – Center of Marine Sciences, Campus de Gambelas, University of Algarve, 8005-139 Faro, Portugal
[2]University of Bremen, 28359 Bremen, Germany
[3]Alfred-Wegener-Institut Helmholtz-Zentrum für Polar und Meeresforschung, Biologische Anstalt Helgoland, 27483 Helgoland, Germany
[4]Department of Environmental Science, Policy, and Management, Mulford Hall, University of California, Berkeley, Berkeley, CA 94720, USA

ABSTRACT: Dams alter the natural dynamics of river inflow, disrupting biological processes in downstream ecosystems, as observed in the Guadiana estuary (SW Iberian Peninsula, Europe). Here, significant interannual fluctuations in the densities of jellyfish occur during summer, likely due to changes in winter river discharge. Therefore, this study aimed to quantify the relationship between winter river inflow and the abundance of jellyfish in the Guadiana estuary. In addition, the budding and growth of *Aurelia aurita* polyps, one of the bloom-forming species present in the estuary, were determined at different combinations of constant temperature and salinity. The response of polyps and ephyrae to short-term, low-salinity pulses was also quantified. Maximum winter river discharge and maximum abundance of estuarine medusa (bloom indicator) showed a significant negative correlation. Under constant conditions, polyps showed increased mortality when water temperature was higher than 23°C and salinity was lower than 23, and died when exposed to a short-term, low-salinity pulse (≤3). After exposure to freshets, polyp budding and feeding rates decreased by 69% and 32%, respectively, when salinity reached values as low as 10. Ephyrae died when salinity was lower than 10, and feeding rates decreased by 88% when salinity was 17, compared with full marine conditions. In conclusion, winter freshwater discharge may regulate the strength of estuarine jellyfish blooms, impairing the survival or condition of polyps and ephyrae during late winter or early spring. River basin managers should consider the prescription of freshets to prevent jellyfish blooms from disrupting ecosystem services (e.g. fisheries, tourism).

KEY WORDS: River flow management · Freshets · Polyps · Ephyrae · Estuary

INTRODUCTION

Episodic jellyfish blooms are frequent and in many cases unpredictable, particularly in coastal areas and semi-enclosed water bodies (Mills 2001, Purcell et al. 2007). Current management practices aiming to preserve essential ecosystem services, such as fisheries and beach recreation (Purcell 2005, Purcell et al. 2007), attempt to mitigate the problems caused by jellyfish outbreaks (e.g. early warning systems, employment of selective fishing gears, consumption of jellyfish products) (Richardson et al. 2009, Boero 2013) rather than prevent the onset of a bloom.

*Corresponding author: mchichar@ualg.pt
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Jellyfish dynamics are governed by natural phenomena and also by anthropogenic-induced drivers, such as global warming, eutrophication, overfishing (Richardson et al. 2009, Purcell 2012, Boero 2013), ocean sprawl (Purcell 2012, Boero 2013, Duarte et al. 2013), and alterations in the flow of freshwater (Xian et al. 2005, Purcell et al. 2007). Global warming augments jellyfish growth and reproduction rates (Purcell et al. 1999, 2009, Liu et al. 2009, Holst 2012, Wang et al. 2015), while ocean sprawl provides increasing substrate availability for the benthic stages of many species (Duarte et al. 2013). Eutrophication and productive ecosystems (e.g. estuaries, coastal lagoons) offer enough food for opportunistic feeders such as gelatinous zooplankton (Morais et al. 2015). Eutrophication also provides a competitive advantage for jellyfish due to their resistance to low levels of dissolved oxygen (Purcell & Arai 2001, Purcell et al. 2001, Ishii et al. 2008), while overfishing eliminates competitors and predators (Boero 2013, Marques et al. 2016). Finally, alterations in river discharge caused by dams — i.e. reduction of river inflow, disruption of natural river flow patterns — are likely to increase estuarine jellyfish blooms, but research on this topic is still scarce.

River flow alterations affect the reproduction of estuarine jellyfish (Purcell et al. 1999, 2009), the feeding behavior of polyps (Holst & Jarms 2010), and the settlement of planulae larvae (Conley & Uye 2015) due to shifts in the estuarine salinity conditions. Indeed, it is hypothesized that river flow management influences the fitness of estuarine jellyfish (Morais et al. 2015). Yet, the occurrence of juvenile benthic stages of many species is ill-documented, with existing reports only emphasizing that estuaries and coastal lagoons are important breeding grounds (Lucas 2001, Pagés 2001, Makabe et al. 2014, van Walraven et al. 2016).

Indeed, the formation of jellyfish blooms largely depends on the successful development of their early life stages (Hernroth & Gröndahl 1985, Lucas 2001, Lucas et al. 2012, Makabe et al. 2014). A few reports indicate that reduced freshwater discharge and high-salinity conditions during winter and early spring precede severe jellyfish outbreaks in the following summer (Cargo & King 1990, Xian et al. 2005), while intense river discharge minimizes the formation of blooms. Therefore, we hypothesize that late winter and spring freshwater pulses (i.e. freshets) will decrease the size of the jellyfish population during summer and that the use of freshets may be a potential efficient management practice.

In this context, this study aimed to determine whether acute salinity changes as a result of freshwater pulses can eliminate or decrease summer jellyfish blooms. For this we used 2 approaches: an observational approach and an experimental approach. In the observational approach, we established a relationship between winter river discharge and maximum medusae abundance in the following summer in the Guadiana estuary (SW Iberian Peninsula, Europe) using a zooplankton time-series dataset of the occurrence of gelatinous species in the estuary using 10 years' worth of data. We focused on *Blackfordia virginica*, *Aurelia aurita*, *Obelia* sp., *Bougainvillia muscus*, *Maeotias marginata*, and *Catostylus tagi*, as these comprised the most important species in the Guadiana estuary (Muha et al. 2012, 2017). In the experimental approach, we used the moon jelly *Aurelia aurita* (Linnaeus, 1758) (Cnidaria, Scyphozoa) as a model species because it forms blooms in many estuarine and coastal ecosystems (Mills 2001), including in the Guadiana estuary (L. Chícharo et al. 2009). This approach consisted of a series of experiments aimed at evaluating the effects of constant temperature and salinity as well sudden changes in salinity on (1) survival of polyps and ephyrae, (2) growth and asexual reproduction traits (budding, strobilation, and ephyrae production), and (3) ecophysiological performance traits of ephyrae (feeding and pulsation rate).

The polyps of *A. aurita* reproduce asexually by budding or strobilation, which produces ephyrae that grow into adult medusae. Temperature and salinity affect the performance and survival of polyps and ephyrae (Purcell 2005, Holst 2012, Algueró-Muñiz et al. 2016). Decreasing water temperature favors strobilation (Liu et al. 2009, Holst 2012, Wang et al. 2015), while a decrease in salinity reduces strobilation (Sokołowski et al. 2016). Budding and growth of ephyrae and medusa increase under higher water temperatures, but the influence of salinity on budding and growth is controversial (Willcox et al. 2007, Han & Uye 2010, Wang et al. 2015). Nonetheless, most experimental studies have been conducted using relatively narrow salinity ranges (12–36) or gradual salinity changes (e.g. Willcox et al. 2007, Holst & Jarms 2010). Such treatments represent long-term changes in salinities but do not mimic sudden short-term freshwater pulses (freshets) into estuaries (L. Chícharo et al. 2006, M. A. Chícharo et al. 2006). Estuaries experience such sudden changes over short periods of time, either due to rainfall and subsequent river run-off or by sudden and intentional water discharge from dams (Cloern & Nichols 1985, Morais et al. 2012).

MATERIALS AND METHODS

Observational approach: freshwater discharge and abundance of medusae in the Guadiana estuary

The Guadiana estuary is located in the southwestern Iberian Peninsula and drains into the Gulf of Cádiz (Atlantic Ocean) (Fig. 1). The estuary has an average depth of 6.5 m, occupies an area of 22 km^2, and the tidal amplitudes range from 1.3 to 3.5 m. This is a mesotidal estuary with a tidal wave that propagates inland for at least 70 km. River flow varies within and among years due to variations in rainfall and river flow management (Morais et al. 2009, 2012, Garel & Ferreira 2015). The middle and lower estuary reach low-salinity conditions under average inflow conditions, and salinity can reach zero in the lower estuary during floods or freshets (Wolanski et al. 2006, Morais et al. 2009). However, since the completion of the Alqueva Dam in February 2002, which formed the biggest freshwater reservoir in Europe (Fig. 1) (Morais 2008), the river discharge is usually lower than 50 $m^3 s^{-1}$, despite episodic flooding events (e.g. March–April 2013) (Garel & D'Alimonte 2017).

The abundance of medusae were obtained as the total of hydromedusae (e.g. *Blackfordia virginica*, *Obelia* sp.), and also included scyphomedusa ephyrae and juveniles (e.g. *Aurelia aurita* and *Catostylus tagi*, the most abundant Scyphomedusae in the area) (Muha et al. 2012, 2017). They were collected as a contribution to the International Group for Marine Ecology Time Series (IGMETS), in the Guadiana lower estuary, southwest Iberian Peninsula (O'Brien et al. 2016; www.st.nmfs.noaa.gov/copepod/time-series/pt-30201/). Sub-superficial (50 cm depth) horizontal plankton tows were carried out with a WP2 net to collect zooplankton samples in 2 downstream stations of the Guadiana River. The monitoring of zooplankton in this estuary began in 1997, motivated by the need to have a reference description of communities before the start of the Alqueva Dam operations in February 2002. This dam is located ~140 km from the river mouth. No zooplankton samples were collected between 2003 and 2008 due to lack of funds. Excluding this period, 2 stations were sampled monthly in the estuary during summer, one in the middle estuary (brackish zone) and another in the lower estuary (marine zone; seasonal variation of temperature from 13 to 24°C, and salinity from 0 to 36) (more detailed information is available at the IGMETS website).

Freshwater discharge into the estuary was measured at Pulo do Lobo hydrometric station (ref. 27L/01H; 37° 48′ 11″ N, 7° 37′ 59″ W) and retrieved from SNIRH (2016). This hydrometric station is located in the Guadiana River at 85 km from the river mouth (Fig. 1).

The relationship between winter river discharge (maximum value of freshwater inflow in $m^3 s^{-1}$ measured between January and March) and maximum medusa density (in individuals [ind.] m^{-3}) during summer (from July to September 2001, 2002, and 2009–2016) was investigated using a power function model. A significance level of 0.05 was assigned *a priori*. All analyses were done using R (R Development Core Team 2016).

Experimental approach

Aurelia aurita was used as a model species, because it is very difficult to work experimentally with the other common species present in the estuary, as there are no established protocols. Nevertheless, due to the difficulty in finding *A. aurita* polyps in the Guadiana estuary at the time of the experiments, polyps were obtained from different sources. For the

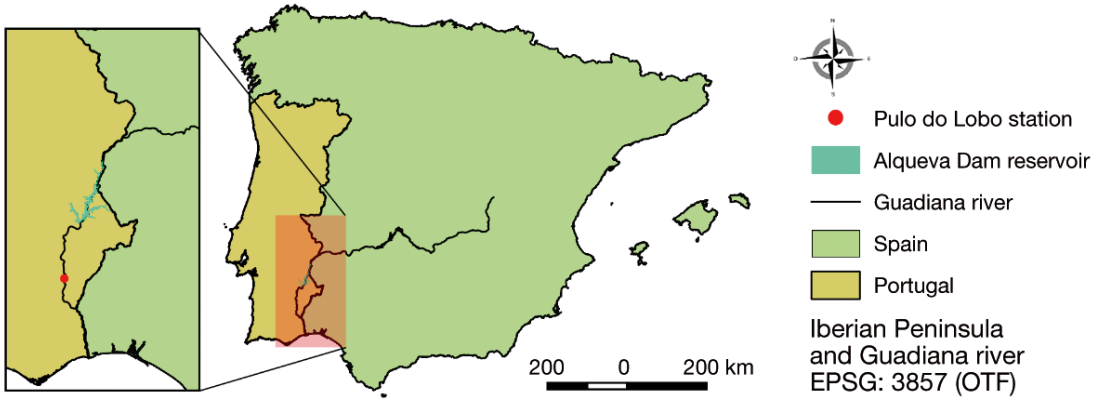

Fig. 1. Location of the Guadiana River, Alqueva Dam reservoir, Pulo do Lobo station and Guadiana estuary in the Iberian Peninsula (Europe)

first set of experiments, performed under different constant temperature and salinity conditions, adults of *A. aurita* medusae were collected offshore of Helgoland (Germany, North Sea) and their planulae larvae raised in a polyp culture. For the other experiments, which simulated freshwater pulses, polyps of *A. aurita* also originating from the North Sea were obtained from ZOOMARINE, a public aquarium in southern Portugal.

Polyps: somatic growth, survival, and budding under constant temperature and salinity

Polyps were kept in darkness and fed ad libitum with a mixture of different stages of *Artemia franciscana* (Artemiidae, Branchiopoda), which was collected 48 h after hatching. Six hundred polyps were reared on 60 plastic Petri dishes (12 cm^2, 10 polyps per dish) in seawater at salinity 35 and at 10°C in a temperature-controlled room. Each Petri dish (experimental unit [EU]) was then transferred to a 700 ml beaker with water at one of 6 different salinities (35, 31, 27, 23, 19, 15) over a temperature-table set at 10 different temperatures ranging from 7.9 ± 0.2°C to 25.1 ± 0.1°C (Table 1, Fig. 2). The petri dish floated on the water surface with the polyps upside down. The experiment ran for 19 d. They were fed ad libitum every 2 to 3 d with brine shrimp. The polyps were allowed to feed over 12 h, and then the water was exchanged to remove uneaten nauplii.

Table 1. Temperatures and salinities (mean ± SD) of the experimental treatments to assess optimal conditions for polyps (see Fig. 2). The values correspond to the rows and columns of the temperature table, respectively

Temp. (°C)	Category	Salinity	Category
7.9 ± 0.2	Low	15.1 ± 0.4	Low
9.6 ± 0.2		19.2 ± 0.4	
12.0 ± 0.1			
13.5 ± 0.2	Intermediate	23.2 ± 0.8	Intermediate
16.0 ± 0.1		27.2 ± 0.6	
17.4 ± 0.1			
19.4 ± 0.1		31.2 ± 0.6	High
20.9 ± 0.2		35.3 ± 0.7	
23.4 ± 0.2	High		
25.1 ± 0.1			

The size of each polyp was measured (ranging from 1.5 to 2.2 mm) on Days 0, 7, 13, and 19, after being gently poked with a blunt needle until it fully contracted (Lesniowski et al. 2015). A photograph was then taken on the oral side to measure its diameter (±0.1 mm). Polyps that detached from the EU, or died, were counted during the experiment to quantify the survival rate.

The buds per EU were counted to quantify asexual reproduction, through budding, on Days 13 and 19. Buds were gently removed from the EU to maintain the same number of polyps in each treatment and replicate, and to prevent density-dependent effects on polyps' growth. Buds that were not fully developed were not counted.

Somatic growth rates (G_r) were calculated by assuming an exponential growth of polyp size (diameter, mm) following Eq. (1):

$$G_r = [\ln(d_t) - \ln(d_0)] \times \Delta t^{-1} \quad (1)$$

where d_t is the diameter at the end of an experiment, d_0 is the initial diameter, and Δt is the number of experimental days.

The influence of temperature, salinity, and their interaction on polyp growth and budding was assessed using a generalized linear mixed model. Data exploration suggested a quadratic relationship between growth rate and salinity; therefore, for this model, salinity was squared. Polyp size at the beginning of the experiment was included as a random variable, as initial size may have an influence on growth. Similarly, for the

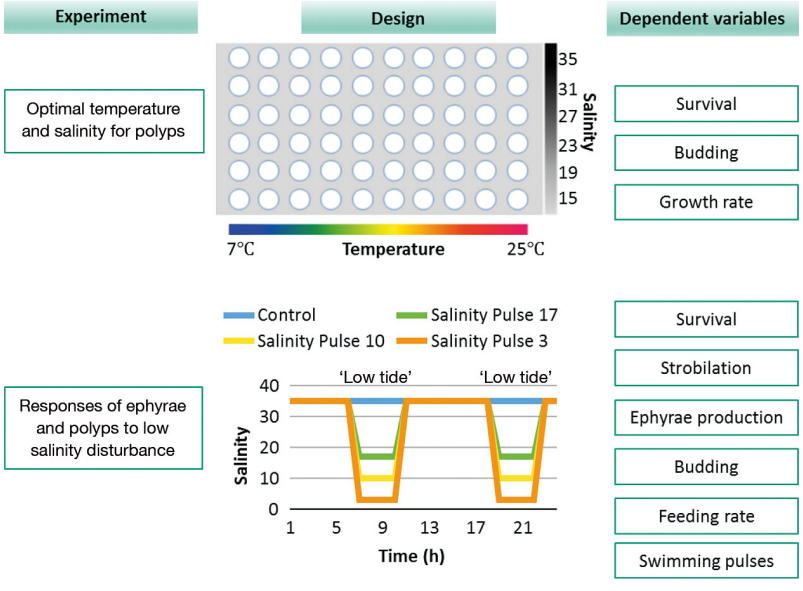

Fig. 2. Treatment design and dependent variables for the polyps in the optimal temperature and salinity experiment, and the salinity pulse experiment

budding rate model, the initial number of buds (Day 13) and polyp size (Day 7) were also considered random variables. A logistic regression was used to model the survival of polyps (proportional data), using a binomial distribution. This procedure deals with survival proportions as probabilities for each case (i). In logistic regression, the logarithmic odds of an event are modelled as a linear function of the explanatory variables (Zuur et al. 2009):

$$\ln(O_i) = \ln[P_i / (1 - P_i)] \quad (2)$$

where O_i are the odds, P_i the probability of success, and $\ln(O_i)$ a linear combination of the explanatory variables.

Variance was considered dependent on temperature and salinity for all models to avoid heteroscedasticity (Zuur et al. 2009). The polyps of treatment '12°C + salinity 19' were not considered in the analysis because their initial diameter was substantially larger (3 mm) than the others (1.5–2.2 mm; mean = 1.8 mm) for unknown reasons.

Polyps: survival, reproduction, and feeding after freshwater pulses

Polyps were kept in aerated seawater at 21°C and fed ad libitum twice a week. Polyps with a diameter ranging from 1.9 to 2.3 mm were gently detached from the substrate, placed into 12 ml Petri dishes, fed with brine shrimp nauplii, and kept for 6 d in beakers containing 150 ml of seawater at 21°C to settle in the EU. All beakers were kept aerated.

Water temperature was set to decrease from 21 to 10°C over 5 d to stimulate strobilation, and then kept constant at 10°C until the end of the experiment. On Day 5, triplicate EUs (each containing 3 polyps) were subjected to 2 pulses of brackish water (salinities 3, 10, and 17) with a 9 h interval (Fig. 2) to simulate the effect of freshets into estuaries with semidiurnal tide variation. Pulses were simulated by transferring the EU to beakers filled with 150 ml of water at salinities 3, 10, and 17. Immersions lasted for 3 h and were separated by a 9 h interval, during which polyps remained in fully saline (35) seawater. Thus, 12 h elapsed between the beginning of the first and second treatment, which corresponds approximately to the time elapsed between 2 consecutive low-tide periods. Polyps continued being fed ad libitum once a week after freshwater-pulse treatments.

The effect of freshwater pulses on the feeding activity of polyps was determined 5 h after the last pulse. Each EU contained 12 ml of seawater and polyps were fed with brine shrimp nauplii (75 ± 8 nauplii per EU). Three extra EUs containing only brine shrimp nauplii served as the control. Polyps were fed for 1 h and then the remaining nauplii were counted.

The survival of polyps was evaluated at the end of the freshwater-pulse experiment by counting the number of live specimens. Buds produced at Days 5 and 13 after the end of the freshwater-pulse treatment were counted and removed. Polyps started to strobilate 13 d after the end of the freshwater-pulse treatment. The first ephyra was released at Day 29, and the number of ephyrae was recorded every second day until Day 59, the day when the last ephyra was released.

In the low-salinity pulse experiments, the influence of salinity on dependent variables (ingestion rate, budding, number of ephyrae produced) was analysed with ANOVA, followed by a Tukey test with the significance level set at 0.05, after checking for normality (Shapiro-Wilk test) and homoscedasticity (Bartlett test).

Ephyrae: survival, feeding, and swimming after freshwater water pulses

The ephyrae released by polyps were kept mixed in beakers with seawater at 10°C and gently aerated. Twelve 1-d-old ephyrae, 2–3 mm in diameter, were distributed in twelve 3 cm^3 EUs. Similar to the previous experiment, ephyrae were submitted to brackish water pulses (salinities 3, 10, and 17) and to a control treatment in which water was kept at a constant salinity of 35. Three replicates were taken for each treatment and survival was determined in each one.

Twelve other ephyrae, ranging between 2 and 3 mm, were placed in 12 EUs. Six EUs were submitted to double pulses of water with salinity at 17, and the other 6 EUs to double pulses of water with salinity at 35. The number of umbrella beats (contractions) was counted for 1 min (pulsation rate) following the treatments, using a stereomicroscope, with ephyrae from both treatments already immersed in seawater.

Six 2-d-old ephyrae, ranging from 3 to 4 mm, were placed individually in EUs and submitted to 2 water pulse treatments, one with water at salinity 17 and the other at 35. On treatment conclusion, ephyrae were placed in seawater and fed brine shrimp nauplii, and the number of nauplii ingested over 30 min was determined by counting the nauplii inside ephyrae.

In the low-salinity pulse experiments, the influence of salinity on dependent variables (ingestion rate, pulsation rate) was analysed with ANOVA followed by a Tukey test with the significance level set at 0.05, after checking for normality (Shapiro-Wilk test) and homoscedasticity (Bartlett test).

RESULTS

Abundance of medusae in the Guadiana estuary and freshwater discharge

The maximum abundance of medusae in the Guadiana estuary varied between 1 ind. m^{-3} (2013, 2014) and 60 ind. m^{-3} (2016). In 2016, the maximum winter flow was 22 m^3 s^{-1} and it reached 790 m^3 s^{-1} in 2013. Whenever winter flow was higher than 400 m^3 s^{-1}, the abundance of medusae was lower than 8 medusae m^{-3} with an average of 3.0 ± 3.4 medusae m^{-3}. The abundance of jellyfish in the Guadiana estuary showed a significant inverse relationship with average river flow of the previous winter (R^2 = 0.744, $p < 0.05$; Fig. 3, Table 2).

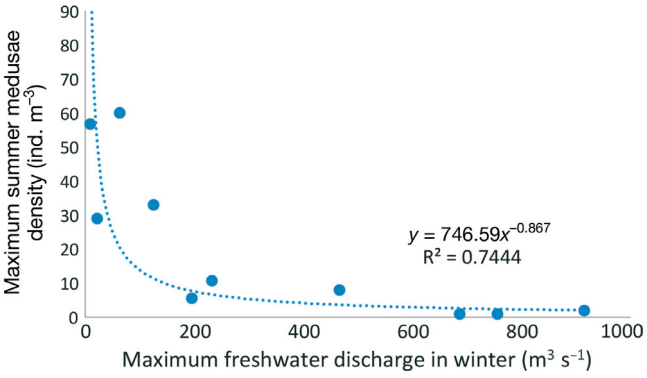

Fig. 3. Maximum winter freshwater flow into the Guadiana estuary versus density of the peak of jellyfish during the summer months July, August, and September in 2001–2015. There is a significant inverse relationship between these 2 variables (p = 0.03) (Table 2)

Table 2. Linear model results for the correspondence between winter (January–March) maximum river discharges (log freshwater flow) into the Guadiana estuary, and peak jellyfish density during the following summer (July–September), based on data from 2001, 2002, and 2009–2016 (see also Fig. 3)

	Estimate	SE	*t*	p
(Intercept)	5.7644	0.9509	6.062	<0.001
Flow	−0.8069	0.2064	−3.909	<0.01

Polyps: somatic growth, survival, and budding under constant temperature and salinity

The growth of *Aurelia aurita* polyps topped at 0.046 d^{-1} (salinity 23, 19°C) and was significantly affected by temperature, salinity, and their interaction ($p < 0.001$; Table 3, Fig. 4A). Polyps grew faster (>0.035 d^{-1}) in treatments with water temperature ranging between 11 and 19°C and between salinities 19 and 35 (Fig. 4A). The strongest shrinking case (−0.033 d^{-1}) was recorded at salinity 15 and 25°C (Fig. 4A). Shrinking was observed in treatments with temperatures higher than 25°C and salinities ≤27 (27, 25, and 19). The smallest polyps (1.02–1.96 mm) were also found in these treatments at the end of the experiment, and they showed a degeneration in general shape and tentacles.

Polyp survival varied between 20% and 100%, reaching lowest values under the most extreme treatment (salinity 15, 25°C). There was a statistically significant effect of salinity, temperature, and their interaction on survival rate ($p < 0.001$; Table 3). Survival was lower at low salinities and warm temperatures (Fig. 4B). For instance, 80%, 70%, and 40% of the polyps died in the 25°C treatments with salinity 15, 19, and 23, respectively.

The budding rate varied between 0 and 2.37 buds d^{-1} EU^{-1} and was significantly affected by temperature, salinity, and their interaction ($p < 0.001$; Table 3). Budding occurred at intermediate and higher temperatures (13.5–23.0°C), and polyps produced the largest amount of buds between salinities 19 and 27 (Fig. 4C).

Polyps: survival, reproduction, and feeding after low-salinity pulses

In the acute salinity exposure experiment, 100% of polyps died under a salinity pulse of 3, while 100% of polyps survived salinity pulses of 10, 17, and 35. The rate of buds produced in the following 13 d varied between 0.025 buds $polyp^{-1}$ d^{-1} (salinity 10 pulse treatment) and 0.230 buds $polyp^{-1}$ d^{-1} (salinity 17 pulse treatment), and was statistically significantly lower under salinity pulses of 10, with an average of 0.042 ± 0.024 buds $polyp^{-1}$ d^{-1} (Tukey test, $p < 0.01$), and higher under salinity pulses of 17, with an average of 0.196 ± 0.024 buds $polyp^{-1}$ d^{-1} (Tukey test, $p < 0.01$), when compared with the

Table 3. Full factorial experiment. ANOVA results for (1) the mixed linear model for the growth rate of polyp diameter (d^{-1}) over 19 d with initial size as random variable, temperature (10 categories) and salinity (polynomial of order 2) as independent variables, and their interaction; (2) the generalized linear model (model: quasi; link: log) of polyp survival with temperature (10 categories) and salinity as independent variables, and their interaction; and (3) the linear mixed model of polyp budding with number of buds on Day 13 and polyp size on Day 7 as random variables, temperature (10 categories) and salinity as independent variables, and their interaction with variance dependent on temperature and salinity (varPower(form=~Temperature|Salinity))

Dependent variable	Factors and interaction	df	*F*	p
Growth rate	Temperature	1	2507	<0.0001
	Salinity^2	9	17.6×10^{10}	<0.0001
	Temperature × (salinity^2)	9	52	<0.0001
Survival	Temperature	9	13.4	<0.0001
	Salinity	1	3.8	<0.0001
	Temperature × salinity	9	8.0	<0.0001
Budding rate	(Intercept)	1	6	<0.05
	Temperature	9	9.9×10^6	<0.0001
	Salinity	1	50	<0.0001
	Temperature × salinity	9	12	<0.0001

control treatment (constant salinity of 35; (0.136 ± 0.012 buds $polyp^{-1}$ d^{-1}) (Table 4, Fig. 5A).

The number of strobilae varied between 1 strobila (salinity 17 treatment) and 3 (salinities 10 and 35 treatments), and there was no statistically significant difference between any of the salinity pulse treatments (p = 0.87; Table 4, Fig. 5B). The polyps subjected to salinity pulses of 10 and 35 produced an average of 2.0 ± 0.8 strobilae over the experiment (59 d), while those subjected to salinity pulses of 17 produced an average of 1.67 ± 0.47 strobilae.

The number of ephyrae produced per polyp during 29 d varied between 8 (salinity 17 treatment) and 15 (salinity 35 treatment) ephyrae $polyp^{-1}$. The average of ephyrae produced in

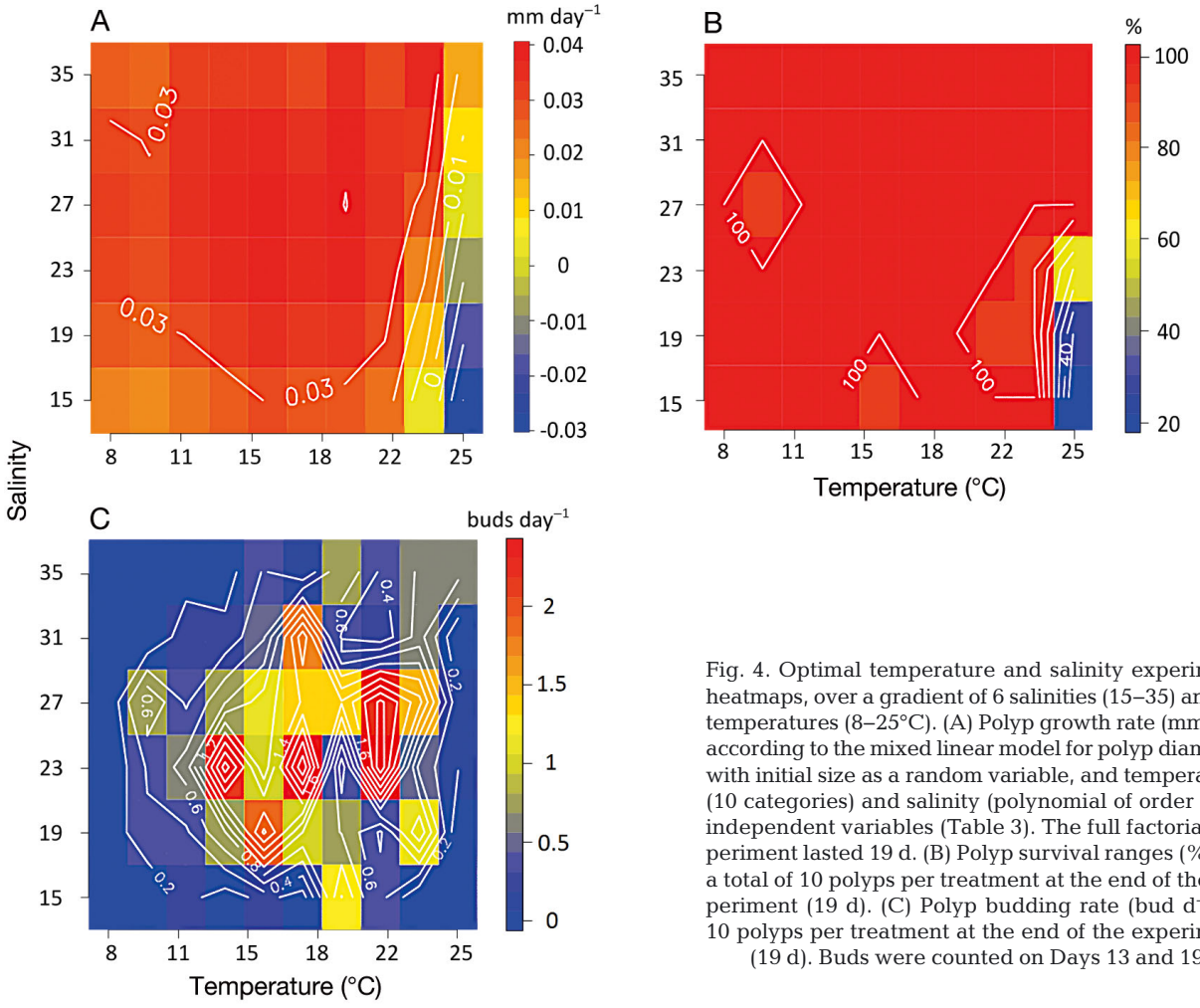

Fig. 4. Optimal temperature and salinity experiment heatmaps, over a gradient of 6 salinities (15–35) and 10 temperatures (8–25°C). (A) Polyp growth rate (mm d^{-1}) according to the mixed linear model for polyp diameter with initial size as a random variable, and temperature (10 categories) and salinity (polynomial of order 2) as independent variables (Table 3). The full factorial experiment lasted 19 d. (B) Polyp survival ranges (%) for a total of 10 polyps per treatment at the end of the experiment (19 d). (C) Polyp budding rate (bud d^{-1}) of 10 polyps per treatment at the end of the experiment (19 d). Buds were counted on Days 13 and 19

Table 4. Freshwater pulse experiments. Linear model results of budding (including post-hoc test), number of strobilae and ephyrae production, polyp feeding rate, ephyrae feeding rate, and pulsation rate after acute salinity variation

Dependent variable	Factor(s)	df	*F*	p
Budding rate	Salinity	2	23.23	<0.01
	17–10			<0.01
	35–10			<0.01
	35–17			<0.01
Number of strobilae	Salinity	2	0.143	0.87
	17–10			0.89
	35–10			1
	35–17			0.89
Number of produced ephyrae	Salinity	2	0.545	0.606
	17–10			0.85
	35–10			0.88
Polyp feeding rate	Salinity	6	6.146	<0.05
	Intercept			<0.01
	17			<0.01
	35			<0.05
Ephyrae feeding rate	Salinity	4	24.5	<0.01
Ephyrae pulsation rate	Salinity	10	14.37	<0.01

the control treatment (salinity 35) was 12.1 ± 2.6 ephyrae polyp^{-1}, while in the treatments with salinity pulses of 17 and 10 it was 9.7 ± 1.4 ephyrae polyp^{-1} and 10.7 ± 2.4 ephyrae polyp^{-1}, respectively. There was no significant difference among treatments ($p = 0.32$; Table 4, Fig. 5C).

The ingestion rate of polyps varied between 10.3 (salinity 10 treatment) and 24.7 (salinity 17 treatment) *Artemia* nauplii h^{-1} polyp^{-1}. The ingestion rate was significantly lower in the treatment with a salinity pulse of 10 (15.1 ± 3.2 *Artemia* nauplii h^{-1} polyp^{-1}) than the one observed in the treatment with a salinity pulse of 17 (22.9 ± 5.0 *Artemia* nauplii h^{-1} polyp^{-1}, $p < 0.01$) and control treatment (22.1 ± 4.7 *Artemia* nauplii h^{-1} polyp^{-1}, $p < 0.05$) (Table 4, Fig. 5D).

Ephyrae: survival, feeding, and swimming after brackish water pulses

All ephyrae survived in the salinity 17 pulse and control (constant salinity of 35) treatments, while 0% survived in the salinity 3 and 10 pulse treatments. Ephyrae ingestion rate varied significantly ($p < 0.01$) between the salinity 17 pulse treatment (0.3 ± 0.0 *Artemia* nauplii 30 min^{-1}) and the control (2.7 ± 1.4 *Artemia* nauplii 30 min^{-1}) following the salinity pulse treatment (Table 4, Fig. 5E). The ephyrae pulsation rate varied between 8 (salinity 17 pulse treatment) and 80 (control) beats min^{-1}. Ephyrae pulsation rate was significantly lower in the salinity 17 pulse treatment, with an average of 17.8 ± 3.7 beats min^{-1} compared with the control (54.7 ± 6.9 beats min^{-1}) ($p < 0.001$; Table 4, Fig. 5F).

DISCUSSION

Freshwater discharge and occurrence of medusae in the Guadiana estuary

Our correlational study revealed that years with higher freshwater discharge during winter and spring are correlated with lower jellyfish densities (scypho- and hydromedusae) during the following summer in the Guadiana estuary, with scyphomedusae being rare in years of high spring precipitation and low-salinity conditions. This estuary is characterized by sudden winter and early spring freshets, due to periods of intense rainfall and dam management, which results in abrupt salinity variations in the estuary, reaching zero values in the downstream water column (M. A. Chícharo et al. 2006, L. Chícharo et al. 2009, Garel & D'Alimonte 2017). These freshets probably function as a stress factor for jellyfish (Cargo & King 1990, Decker et al. 2007, Pereira et al. 2014). A similar correlation was found for *Chrysaora quinquecirrha* in Chesapeake Bay (Cargo & King 1990). Also, jellyfish blooms were linked with high-salinity conditions in the mouth of the Yangtze River, since the intrusion of marine water into the estuary lasts longer and extends further upstream after the construction of the Three Gorges Dam (Xian et al. 2005).

In the Guadiana estuary, winter river discharge is completely dependent on rainfall events and dam management (Garel & D'Alimonte 2017). The Alqueva Dam main policy is to store water for irrigation and hydropower production (Morais 2008). Therefore, sudden freshets into the estuary are not frequent, and mostly linked to periods of intense precipitation which occasionally force dam managers to open the gates (e.g. in March–April 2013). At present, Guadiana's summer river flow is generally higher than in the years preceding the construction of the Alqueva Dam as the enforcement of a minimum river flow threshold and summer flow conditions disabled the occurrence of nuisance cyanobacteria blooms (Domingues et al. 2014), but are insufficient to induce the collapse of summer jellyfish bloom. Therefore, a potential effective way to tackle the problems posed by summer jellyfish blooms is to

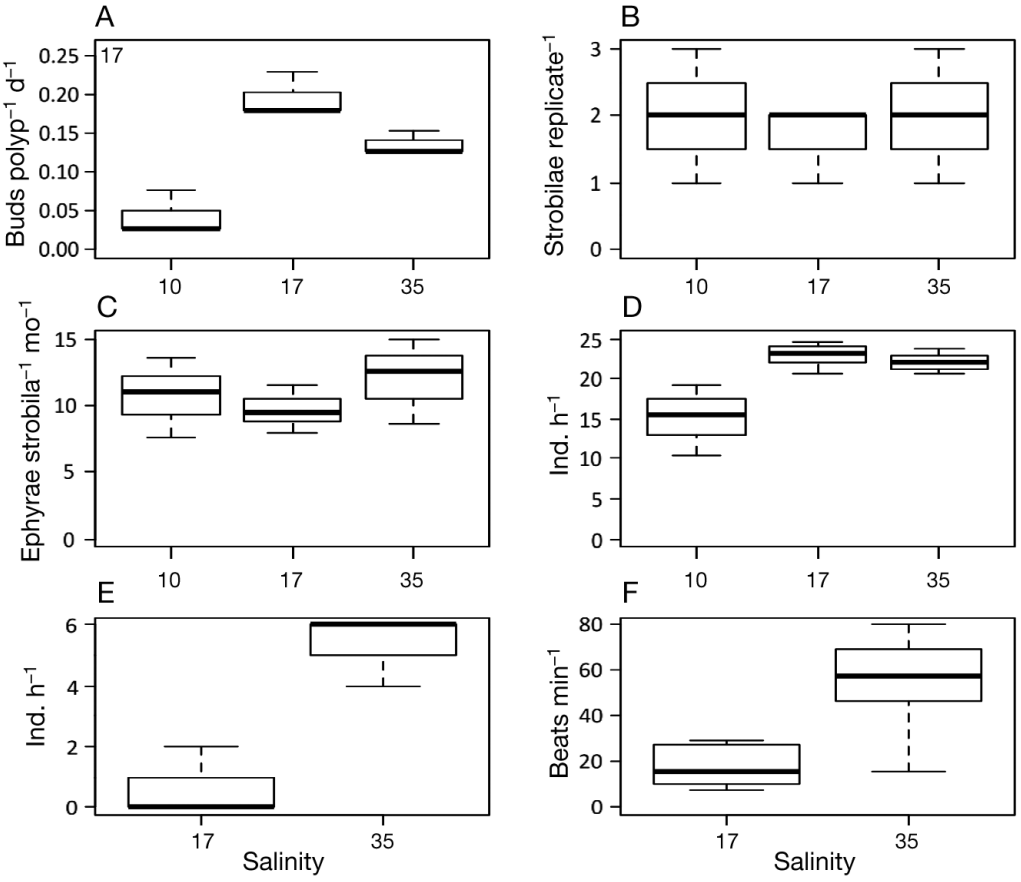

Fig. 5. Boxplots of each tested variable in the salinity pulse experiment (Table 4). Bar: median (50th percentile); box: 25th–75th percentiles; whiskers: 10th–90th percentiles. (A) Budding rate per polyp (buds polyp^{-1} d^{-1}) during 13 d after salinity pulses (n = 3). (B) Number of strobilae in a total of 3 polyps in each replicate after 1 mo (strobilae replicate^{-1}) of salinity pulses (n = 3). (C) Number of ephyrae produced per strobila. The number of released ephyrae was counted during 1 mo (n = 3). (D) Polyp feeding rate (ind. h^{-1}) after acute salinity variation (n = 3). (E) Ephyrae feeding rate (ind. h^{-1}) on *Artemia salina* nauplii after acute salinity variation (n = 3). (F) Ephyrae pulsation rate (beats min^{-1}) after acute salinity variation (n = 6)

manage river flow during late winter to impair the development of jellyfish polyps.

Experimental response of polyps and ephyrae to salinity and temperature changes

The individuals of *Aurelia aurita* used in the experimental component of our study did not originate from the Guadiana estuary, because of limitations in the availability of local specimens. Obviously, this precludes strong statements on what exactly drives the dynamics in the Guadiana estuary. However, by studying organisms from different regions, we increase the generality of our conclusions, which were never aimed to be site specific. This also means that we will refrain from discussing exact values of temperature and salinity that affect growth and asexual reproduction of the organisms as they will probably be population-specific. For example, it could be the case that organisms taken from the North Sea with full marine salinities have lower tolerance for lower salinities and lower temperature optima, than those originating in warmer estuaries. However, our main interest in this study was the effect of salinity, and the likelihood that polyps of the gelatinous zooplankton collected in the North Sea in fact originate from inshore areas or estuaries is very large, as natural hard substrate in the open North Sea is scarce. For example, nearly all of the polyp samples of *A. aurita* collected by van Walraven et al. (2016) were from nearshore. They observed no polyps in the open North Sea, except those taken from wrecks on the Dogger Bank. Moreover, as a result of the planktonic lifestyle of medusae and ephyrae, combined with the prevailing currents in the North Sea, adult individuals caught off Helgoland will certainly have been produced somewhere else, most likely in the estuar-

ies much farther south. Despite the current uncertainty about the complex *Aurelia* described for the Mediterranean sea (Scorrano et al. 2016), the polyps used in the experimental approach had their source from the North Sea and were considered to be from *A. aurita* species.

Our experimental data support the link between pulses of freshwater and impaired performance of *A. aurita* asexual reproduction. Temperature, salinity, and their interaction significantly affected the survival, ecophysiological performance (i.e. feeding rate and swimming ability), and budding of *A. aurita* early life stages. The polyps used displayed reduced feeding under low salinity compared with intermediate and high salinity (35) conditions. Reduced feeding might result from a physiological reaction or osmotic stress, but also from a degeneration of tentacles, as observed for polyps from the Baltic Sea (Holst & Jarms 2010). Although the degeneration of tentacles was not evaluated in this study, and since salinity-induced morphological changes have the potential to impact feeding (Holst & Jarms 2010), this will have consequences for size (Spangenberg 1964, Gong 2001, Willcox et al. 2007), strobilation (Thiel 1962, Purcell et al. 1999), and budding (Han & Uye 2010), and ultimately the bloom dynamics of estuarine gelatinous zooplankton.

Polyps struggled under chronic salinity conditions below 15 and acute salinity variations falling below 10. The growth of polyps was impaired in treatments with the lowest salinity and highest temperature, and they grew best under low and intermediate temperatures (see also Han & Uye 2010, who observed the same patterns with individuals taken from areas with much higher temperatures), and at intermediate to high salinities. Thus, polyps with the highest growth rates are likely to be found in areas with average salinity higher than 19 and intermediate temperatures. These conditions would also maximize the production of ephyrae because the size of polyps determines the number of ephyrae produced (Spangenberg 1964). Thus, freshets resulting in low salinities during prolonged periods will negatively affect the growth of polyps and production of ephyrae.

The higher budding rates were observed at intermediate to high temperatures (13.5–23.4°C) and intermediate salinities (19–27) in the full-factorial experiment, as well as in the acute salinity experiment (17). Indeed, Kiel Bight (Baltic Sea) polyps also produced more buds in intermediate conditions (18) than in lower-salinity conditions (12) (Sokołowski et al. 2016). Similarly, Tapong Bay (Taiwan) polyps also produced more buds in warmer (20°C) than colder conditions (10°C) (Liu et al. 2009). Several other species also showed highest budding production under intermediate salinity conditions — between 10 and 20 for *Chrysaora quinquecirrha* (Scyphozoa: Pelagiidae) (Purcell et al. 1999), and between 20 and 27 for *Nemopilema nomurai* (Scyphozoa: Rhizostomeae) (Dong et al. 2015).

The number of strobilating polyps and ephyrae were not significantly different within salinity pulses. These results contradict those of Holst & Jarms (2010) and Purcell et al. (1999) who found higher strobilation of *A. aurita* and higher production of *C. quinquecirrha* ephyrae under higher-salinity conditions, respectively. The discrepancy with other studies is likely due to a period with no low-salinity conditions between the freshet treatment and strobilation, which allows polyps to recover and strobilate normally without being affected by the freshet. Our *A. aurita* polyps started the strobilation process 13 d after treatment, which suggests that a 13 d period without salinity variation allows polyps to recover and strobilate normally; however, this period is likely to differ between populations (Pascual et al. 2015).

As expected, *A. aurita* ephyrae were more sensitive to salinity variations than polyps. Ephyrae withstood the salinity 17 pulse treatment without obvious impact, but their survival decreased below this threshold. In contrast, polyps from the same population survived freshwater pulses down to salinity 10. Moreover, feeding and swimming activity of ephyrae were negatively affected by salinity 17, while polyps still performed well under salinity 10. This difference suggests that polyps, as they are sessile and have a longer life-expectancy than ephyrae, need broader physiological tolerance than ephyrae, which as a result of their planktonic lifestyle typically face fewer fluctuations during their life. It also provides some evidence that the typical habitat of polyps is possibly more inshore in lower-salinity environments compared with ephyrae. The strategy of polyps to reproduce by budding during moderate freshwater disturbance periods and by strobilation during reduced discharge (saltier) periods certainly seems optimal to maximize ephyrae recruitment.

Overall, budding and growth of polyps standing stock are expected to be lowest during colder periods and under lower-salinity conditions. Moreover, ephyrae were more sensitive to freshets. Therefore, freshwater pulses controlled by dams may significantly reduce the viability of estuarine polyps and particularly of ephyrae during late winter and early spring.

Prescription of freshwater pulses to control summer jellyfish blooms

Freshwater pulses controlled by dams could be used as an ecohydrological management tool to control or mitigate nuisance blooms in estuarine ecosystems. Such a management approach would produce positive changes in downstream ecosystems by mitigating the formation of jellyfish blooms and by eliminating protistoplankton and cyanobacteria blooms while maintaining diversity and ecosystem services (M. A. Chícharo et al. 2006, Morais et al. 2012). Freshets should be applied while polyps are budding or preceding strobilation. Timing and intensity of freshets are certainly species- and site-specific. However, based on this study, we suggest that freshets must be implemented during late winter in temperate regions to match with the period preceding strobilation and to avoid impairing the nursery function of estuaries for fish species during spring (Faria et al. 2006). It must be stressed that though intermediate salinity conditions (17) impair ephyrae performance, it will enhance budding and thus increase the population density of polyps. Potentially, this larger polyp population will produce jellyfish blooms during stable and high-salinity conditions. Thus, river flow management must include continuous field assessment of populations before the prescription of freshets.

CONCLUSIONS

Freshwater pulses are likely a promising management tool to control estuarine jellyfish blooms, as suggested by the observational and experimental evidence gathered in this work. In the case of moon jelly *Aurelia aurita*, the most efficient way to control estuarine summer blooms would be to impair the survival or condition of polyps and ephyrae during late winter or early spring. Short-term freshwater pulses controlled by dams, setting low-salinity conditions in the areas colonized by polyps, will impair the growth and survival of polyps and ephyrae. This strategy has the potential to control or minimize estuarine summer blooms of gelatinous zooplankton, and is the first potential management strategy that we are aware of that would prevent nuisance jellyfish blooms, rather than attempt to mitigate the impacts of blooms.

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