Universidade de Lisboa
Faculdade de Ciências
Departamento de Biologia Animal

Impact of global warming on the spawning success, metabolism and embryogenesis of a specialized soft coral-feeding nudibranch, *Armina maculata*

Vanessa Leal Pires

Dissertação

Mestrado em Ecologia Marinha

2012

Universidade de Lisboa
Faculdade de Ciências
Departamento de Biologia Animal

Impact of global warming on the spawning success, metabolism and embryogenesis of a specialized soft coral-feeding nudibranch, *Armina maculata*

Vanessa Leal Pires

Orientada pelo Prof. Doutor Rui Rosa e co-orientada pelo Prof. Doutor Luís Narciso da Faculdade de Ciências da Universidade de Lisboa

Mestrado em Ecologia Marinha

2012

AGRADECIMENTOS

Ao Rui Rosa, meu orientador, pela paciência e disponibilidade constantes, pela alegria e gosto no trabalho e por todos os sorrisos que se partilharam. Foi um privilégio tê-lo como orientador.

Ao meu co-orientador, Luís Narciso, pela partilha de ideias, pela disponibilidade e por todas as vezes que carinhosamente me apelidou de "filhota".

À Marta, obrigada por tudo e por teres estado sempre lá para mim. "Muchas gracias!"

À Filipa, Gisela, Miguel e Katja, pela ajuda preciosa e por tudo o que me ensinaram, e a todos os outros colegas e amigos do Laboratório Marítimo da Guia. Vocês fizeram com que todos os dias de trabalho fossem dias felizes.

Aos meus colegas mestrandos, na certeza de que iremos todos orgulhar o nosso orientador.

Ao Bruno, pela incomparável ajuda em muitas das viagens ao estuário, por ter sempre estado ao meu lado em todas as dificuldades técnicas e psicológicas com que me confrontei neste trabalho e pela paciência inigualável, apoio e carinho constantes. Sem ele teria sido muito mais difícil.

A todos os meus amigos que, apesar de a grande maioria nunca ter visto e não saber o que é um nudibrânquio, partilharam comigo diariamente a alegria e o gosto que tive em fazer este trabalho.

À Didi, por me ter feito companhia, dia e noite, durante as horas intermináveis de escrita.

ABSTRACT

Temperature is considered as one of the most important physical variables controlling the life of marine organisms. Thus, understanding how they will respond to future thermal scenarios is of great relevance. The present study aimed to investigate, for the first time, the spawning success, embryonic development and metabolic rates of a specialized soft coral-feeding nudibranch, *Armina maculata*, under different thermal scenarios, which reflect: i) the present-day average spring (18ºC), early-summer (22ºC) and summer (24ºC) temperatures, and ii) the projected near-future warming (27ºC, +3ºC, IPCC 2007), a temperature that the species already experiences during heat wave events in Sado's estuary. At 22ºC, *A.maculata*'s adults spawned more frequently and the embryos showed higher survival rates (~88%), indicating this temperature as the optimal for the reproduction of this species. On the other hand, the project near-future warming (27ºC) promoted metabolic suppression in adults, i.e., it triggered the shut-down of several biochemical functions. Additionally, under such thermal scenario, spawning events were scarce, ceased after three weeks, feeding intake was absent and the individuals perished after seven weeks of incubation. Furthermore, early ontogenetic stages (blastula, gastrula, trocophore, active veliger, newly-hatched, veliger) showed concomitant negative effects with increasing temperature. Embryonic development was dramatically shortened followed by increased energy expenditure rates. Moreover, egg mass analysis showed visible deleterious effects on embryo's development and survival, with the latter decreasing up to 45%. Hence, the ability of *A.maculata* to overcome the developmental and physiological challenges faced at the projected near-future warming scenario will dictate its long-term survival success.

Keywords: Global warming, Reproduction, Metabolism, Nudibranch, *Armina maculata*

RESUMO

As alterações climáticas emergiram no último século como uma preocupação global. O aquecimento da superfície dos oceanos é uma realidade incontestável e as consequências desta tendência podem ter efeitos catastróficos nas comunidades marinhas. Estudos preveem que a superfície dos oceanos sofra um aumento de 3ºC até 2100 ditando grandes alterações nas características das grandes massas de água, como o aumento do seu nível médio, que causará um forte impacte nas comunidades costeiras, ou mudanças nas correntes, com consequências graves a nível de dispersão larvar e disponibilidade alimentar, por exemplo. Compreender como irão as comunidades marinhas responder às rápidas mudanças nos ecossistemas constitui uma prioridade na investigação científica.

A temperatura é um dos principais fatores físicos que influencia os processos fisiológicos nos organismos marinhos ectotérmicos, tais como taxas de respiração, crescimento, alimentação e reprodução. No entanto, a resposta de cada organismo às variações de temperatura está dependente dos seus limites de tolerância térmica e estágio de desenvolvimento. Além destes limites, a sobrevivência e fitness de um organismo podem estar comprometidos, principalmente numa perspetiva a longo termo. Assim, um organismo exposto prolongadamente a elevadas temperaturas aumenta o seu metabolismo exponencialmente até um nível em que a capacidade respiratória e cardiovascular atingem o seu máximo, seguindo-se um conjunto de reações fisiológicas, tais como, falhas no sistema enzimático, desnaturalização de proteínas e danos membranares que podem resultar na morte do indivíduo.

Embora a ordem Nudibranchia seja a maior dentro dos Heterobranchia (Euthyneura, Nudipleura), poucos estudos reportam o efeito da temperatura na ecologia fisiológica dos nudibrânquios e não existem referências quanto à ecologia de *Armina maculata*. Como seres invertebrados e bênticos, que habitam principalmente as zonas intertidal e subtidal, o efeito do aumento da temperatura pode representar um obstáculo à persistência de muitas espécies. Os nudibrânquios são uma espécie carnívora, alimentando-se de um espectro de seres bênticos e sésseis, que não sejam largamente explorados por outros taxa. Adicionalmente, muitas espécies de nudibrânquios apresentam associações predador-presa, designando-se por espécies estonofágicas, estando intimamente ligados à distribuição e sobrevivência da sua única fonte de

recursos alimentares. Assim sendo, a extinção local de determinada presa pode conduzir paralelamente à extinção da espécie de nudibrânquio que dela depende. Esta condição agrava-se perante um cenário de aquecimento global e, consequentemente, a capacidade de resposta e adaptação dos nudibrânquios deve ser avaliada conjuntamente com a capacidade de resposta de adaptação da sua presa. Num cenário futuro de altas temperaturas, onde é esperado um aumento nas taxas metabólicas dos organismos, uma diminuição nas reservas nutricionais pode resultar em falhas no sucesso reprodutor, já que grande parte da energia de um nudibrânquio adulto é direcionada para a reprodução. Atendendo-se ao fato de que a maioria dos nudibrânquios são espécies semélparas com um ciclo de vida anual, entende-se que o estado nutricional do individuo, bem como, as condições ambientais prevalentes durante o período de copulação, desova e eclosão larvar, estejam intimamente ligados ao sucesso reprodutor da espécie.

Estudos sobre o sucesso reprodutor do nudibrânquio *Adalaria proxima* demonstraram que a temperatura afetava significativamente todas as atividades relacionadas com a reprodução, nomeadamente, periodicidade na colocação de posturas, duração do período de desova e viabilidade das massas de ovos. Assim sendo, compreende-se que, embora os nudibrânquios adultos tenham uma maior plasticidade na resposta às variações anuais de temperatura, o seu investimento reprodutor seja direcionado para um curto e único período de tempo, durante o qual as temperaturas são as ideais para assegurar a sobrevivência da descendência. A maioria das espécies de nudibrânquios possuem uma estratégia reprodutora do tipo “r”, isto é, investem em várias em posturas ao longo do período reprodutor, sendo que cada postura é constituída por milhares de ovos, de forma a superar o efeito da seleção natural sobre a grande variabilidade individual da descendência. Esta estratégia revela-se de especial importância para espécies de nudibrânquios que tenham desenvolvimento larvar planctotrófico.

A capacidade de adaptação das espécies de nudibrânquios às adversidades do aquecimento global, pode ficar comprometida logo nos primeiros estágios de vida. Na verdade, é esperado que estes sejam os mais vulneráveis às mudanças de temperatura, por possuírem uma janela de tolerância térmica muito menor que a dos adultos. Estudos realizados em vários moluscos heterobrânquios, demonstraram que o aumento da temperatura diminuía o tempo de desenvolvimento embrionário, com efeitos negativos concomitantes na sobrevivência e morfologia dos embriões. Adicionalmente, a diminuição do período de embriogénese pode levar a um desfasamento temporal entre

disponibilidade alimentar e eclosão larvar. No futuro, as larvas recém-eclodidas terão uma maior necessidade de alimento, devido às altas taxas metabólicas esperadas, pelo que a falta de recursos poderá comprometer a sua sobrevivência.

A atividade metabólica, por sua vez, pode ser considerada como a função fisiológica de um organismo mais dependente das variações de temperatura. Vários estudos foram realizados na perspetiva de identificar os efeitos das variações térmicas no metabolismo de invertebrados marinhos, comprovando-se que o aumento da temperatura é sempre acompanhado de um aumento nas taxas de consumo de oxigénio. Situações de stress ambiental como extremos de temperatura ou privação de alimento, resultam na ativação de uma resposta de supressão metabólica, em que o organismo diminui os seus gastos energéticos ao máximo, procurando sobreviver até surgirem condições ambientais mais favoráveis. Verifica-se também que a taxa de aumento no consumo de oxigénio é mais significativa nos primeiros estágios de vida de um organismo marinho, bem como em nudibrânquios, corroborando a premissa de que estes estágios são os mais vulneráveis e a sua performance e sobrevivência influenciarão diretamente toda a dinâmica populacional da espécie.

Neste sentido, o objetivo do presente estudo foi avaliar, pela primeira vez, o impacte de um cenário futuro de aquecimento na ecologia fisiológica do nudibrânquio *A.maculata*. Indivíduos adultos da espécie *A.maculata* foram capturados no estuário do Sado (costa oeste de Portugal) e mantidos sob quatro cenários térmicos diferentes, que refletiam: i) a temperatura média atual da primavera (18ºC), início do verão (22ºC) e verão (24ºC) e, ii) a temperatura prevista para o verão sob um cenário futuro de aquecimento (27ºC, +3ºC, IPCC 2007), experienciada já presentemente pela espécie durante ondas de calor no verão, no estuário do Sado. Para se avaliar os efeitos da temperatura na alimentação e reprodução, os indivíduos adultos foram colocados aos pares em aquários individualizados, permitindo a copulação, visto esta espécie ser hermafrodita mas apenas realizar fecundação cruzada, e alimentados *ad libitum* com a sua presa, o octocoral *Veretillum cynomorium*. Após iniciado o período reprodutor, as posturas colocadas foram incubadas nas mesmas condições que os adultos e o seu desenvolvimento foi acompanhado diariamente, verificando-se o efeito do aquecimento na morfologia, sobrevivência e tempo de embriogénese. Por fim, foram medidas as taxas de consumo de oxigénio e determinada a sensibilidade térmica (valores de Q_{10}) desta espécie em adultos e embriões.

Embora fosse expetado que as altas taxas metabólicas verificadas nos adultos a temperaturas elevadas (24, 27ºC) fossem acompanhadas por um aumento na ingestão de alimento, tal não se verificou. Na verdade, as maiores taxas foram verificadas a 18 e 22ºC, onde se verificou também a preferência pela presa *V.cynomorium*. Esta diminuição na procura de alimento pode ser explicada pela ativação de uma resposta de supressão metabólica, que resultou numa diminuição da atividade dos indivíduos e no decréscimo da capacidade respiratória, reprodutora e locomotora, levando à morte dos exemplares, após sete semanas de incubação a 27ºC.

Concomitantemente, o cenário de aquecimento (27ºC) teve consequências devastadoras para esta espécie em vários aspetos da sua reprodução. Os adultos mantidos a esta temperatura apenas colocaram posturas durante as primeiras três semanas de teste, as quais demonstraram deformações morfológicas (ex. variações na cor, diminuição no tamanho das capsulas, embriões e larvas recém-eclodidas, nos últimos estágios da embriogénese) e cujo tempo de desenvolvimento até à eclosão foi significativamente encurtado (~5 dias) e acompanhado por altas taxas de mortalidade embrionária (~55%).

Como esperado, o consumo de oxigénio aumentou linearmente com o aumento da temperatura, verificando-se a premissa de que este aumento seria mais substancial nos embriões do que nos adultos, visto que os estágios iniciais são os mais vulneráveis a variações térmicas.

Por outro lado, os resultados apontam o cenário de 22ºC como a temperatura ótima para a reprodução desta espécie. Elevada frequência de desova associada a altas taxas de sobrevivência embrionária (~88%) e sucesso no desenvolvimento ontogenético, sugerem que esta espécie se reproduza preferencialmente no início do verão.

Os resultados deste estudo demonstraram que a temperatura desempenha um papel crucial no ciclo de vida do nudibrânquio *A.maculata*, determinando vários aspetos das suas respostas fisiológicas a nível alimentar, reprodutivo e metabólico. No entanto, a sobrevivência desta espécie, estará dependente da sua capacidade de adaptação às condições de stress extremo no previsto cenário de aquecimento global.

Palavras-chave: Aquecimento global, Reprodução, Metabolismo, Nudibrânquio, *Armina maculata*

TABLE OF CONTENTS

FIGURE CAPTIONS

Figure 1 - Map of the sampling area, the mouth of Sado's estuary (red circle), Portugal. The polygons indicate the extension of the sampling area (~100,000 m^2).

Figure 2 - *A. maculata'* adult survival rates (A) under four different thermal scenarios during the first seventeen weeks of incubation (B), and in total number of weeks. Values are means ± SD. Different letters represent significant differences.

Figure 3 - Effect of temperature in *A. maculata's* food intake: number of prey items (*Veretillum cynomorium*) eaten by individual per week at 18, 22, 24 and 27ºC (A), and adult of *A. maculata* feeding on the octocoral *Veretillum cynomorium* (B). Values are means of five measurements ± SD. Different letters represent significant differences.

Figure 4 – A) *A.maculata* adult breeding pair copulating (~5-13cm individual l length), and B) adult laying an egg mass.

Figure 5 – The effect of four different thermal scenarios in *A. maculata's* spawning, namely: A) ,mean number of spawning frequency events per week during seventeen weeks of incubation, B)(B),spawning frequency (number of egg masses per week), C(C), and total number of egg masses during seventeen weeks of incubation (per each reproductive pair) (D), during seventeen weeks of incubation per each reproductive pair. Values are mean ± SD. Different letters represent significant differences.

Figure 6 – Egg mass of *A.maculata* nudibranch (A), and a detail of an egg mass, with capsules (77 ± 7.9 μm^3 mean volume) containing embryos (0.34 ± 0.03 μm^3 mean volume) (B) appearance during early-stages.

Figure 7 — Effect of warming in the early (blastula and gastrula) and late (trocophore and active veliger) embryonic stagesontogeny of *A. maculata*, namely: capsule volume (μm^3) (A), embryo volume (μm^3) (B), number of embryos per capsule (C), veliger's length (µm) (D) at four different temperature scenarios. Values are mean ± SD. Different letters (capital letters for late stages; small letters for early stages) and asterisk represent significant differences between temperatures and developmental stages, respectively.

Figure 8 - Percentage of embryo survival at four different temperature scenarios.

Figure 9 – Mean cumulative development time (days ± SD) of *A. maculata* eggs at 18, 22, 24 and 27ºC (A) and the respective percentage of egg mass viability (B). Values are means of five measurements ± SD. Different letters represent significant differences.

Figure 10 – Effect of warming in *A.maculata*'s egg masses: visible deleterious effects in the whole egg mass appearance (A) and, visible deleterious effects in capsules and embryos (B).

Figure 11– Metabolic rates (MO_2, µmol O_2 g^{-1} h^{-1}) of *A. maculata* adults (A), and thermal sensitivity (Q_{10}) of *A. maculata* adults, under four different thermal scenarios. Values are means of twelve measurements ± SD. Different letters represent significant differences. Q_{10} values between 2 and 3 indicate active metabolic regulation; Q_{10} values inferior to 1.5 suggest active metabolic suppression.

Figure 12– Metabolic rates (MO_2, µmol O_2 g^{-1} h^{-1}) of *A. maculata* embryos (A), and thermal sensitivity (Q_{10}) of *A. maculata* embryos, under four different thermal scenarios. Values are means of twelve measurements ± SD. Different letters represent significant differences. Q_{10} values between 2 and 3 indicate active metabolic regulation; Q_{10} values inferior to 1.5 suggest active metabolic suppression.

1. INTRODUCTION

Average global sea surface temperatures are expected to increase up to 3ºC by 2100 (Solomon *et al*., 2007). The expected rapid rate of climate change is predicted to dictate profound impacts on marine ecosystems (Edwards and Richardson, 2004; Thomas *et al*., 2004; Perry *et al*., 2005; Pörtner and Knust, 2007; Rosa and Seibel, 2008; Munday *et al*., 2009; Donelson *et al*., 2011) and questions immediately arise regarding the potential of the species and communities to accommodate to these environmental changes (Bhaud *et al*., 1995).

Temperature is one of the most critical factors impacting biological processes in all life-cycle stages of ectotherm organisms, as it modifies their physiological functions, such as metabolic and growth rates, food requirements and reproductive success (Thompson, 1958; Newell, 1973; Potts, 1983; Smith and Sebens, 1983; Havenhand and Todd, 1988a; Havenhand and Todd, 1988b; Carmona-Osalde *et al*., 2004; Pörtner and Knust, 2007; Woods and Moran, 2008; Pimentel *et al*., 2012; Rosa *et al*., 2012; Vinagre *et al*., 2012). How temperature will influence an organism, depends on the species thermal range (Carmona-Osalde *et al*., 2004) and development stage (Moran and Woods, 2007; Woods and Moran, 2008; Pimentel *et al*., 2012; Rosa *et al*., 2012). Beyond the thermal limit, enzymatic system failures, protein denaturalization, respiratory stress and membrane damages will affect the cardiovascular system (Farkas and Nevenzel, 1981; Reiber and McMahon, 1998) and compromise the overall fitness and survival of the species, especially in a long-term perspective (Pimentel *et al*., 2012).

Although Nudibranchia is the largest order within the Heterobranchia (Euthyneura, Nudipleura) (Jörger *et al*., 2010), few studies have reported the effect of temperature in nudibranch's physiological ecology (Thompson, 1958; Dehnel and Kong, 1979; Potts, 1983; Smith and Sebens, 1983; Havenhand and Todd, 1988a; Sisson, 2002; Watt and Aiken, 2003; Moran and Woods, 2007; Woods and Moran, 2008).

Nudibranchs are all carnivorous, feeding on an array of sessile benthic animals (Thompson, 1976; Bertsch and Johnson, 1981; Todd, 1981, 1983; Wägele and Klussmann-Kolb, 2005). Harris (1973) suggested that many nudibranchs depend on predator-prey associations and in fact, stenophagy is common among nudibranchs (Todd, 1981, 1983; Carrol and Kempf, 1990; Knowlton and Highsmith, 2000). This

specialization might impact species survival under a warming scenario, since the local extinction of a preferred prey could cause a reduction in feeding efficiency, providing too little energy to support the expected higher metabolic rate (Smith and Sebens, 1983). Considering that spawning effort and fecundity are directly related to an adult individual's nutritional state (Turner and Lawrence, 1979), the allocation of energy to reproduction is therefore crucial for a species fitness (Havenhand and Todd, 1988b).

Thompson (1958) demonstrated that the spawning events of the dorid nudibranch *Adalaria proxima* such as, duration of spawning season, periodicity of oviposition and viability of an egg mass, were directly affected by temperature. Thus, although adult nudibranchs, as other benthic invertebrates, are able to tolerate relatively high ambient temperatures (Newell, 1973; Smith and Sebens, 1983) they are only reproductively active during some part of the year to insure survival of progeny (Smith and Sebens, 1983). Thereby, although many other factors (e.g. nutritional state, body size, population density) affect the reproductive output of a given nudibranch species (Havenhand and Todd, 1988b; Turner and Lawrence, 1979; Jones *et al*., 1996), temperature can be a major concern, since the majority of nudibranchs are short-lived and semelparous species (Havenhand and Todd, 1988b; Jones *et al*., 1996). However, in most of the nudibranch species, several spawning events may occur throughout the spawning season (Havenhand and Todd, 1988b; Todd 1979; Jones et al. 1996), as a way to overcome the greater variability of individual offspring survivorship, which can be a consequence of having long-term planktonic larvae (Palmer and Strathmann, 1981; Strathmann, 1985; Havenhand and Todd, 1988b).

Furthermore, early ontogenetic stages also rely on a narrow range of temperatures (Moran and Woods, 2007; Woods and Moran 2008) and as these stages are expected to be the most vulnerable ones, warming may constitute a bottleneck for species survival (Kurihara, 2008; Byrne, 2011). Studies comparing ontogenetic development times at different temperatures have been addressed for several heterobranch molluscs, and a negative correlation between these two parameters was always verified (Thompson, 1966; Lalli and Conover, 1973; Perron and Turner, 1977; Dehnel and Kong, 1979; Todd and Havenhand, 1985; Farfan and Ramirez, 1988). Moreover, temperature increase during nudibranch's embryonic development has been proved to cause deleterious effects on embryo's survival and hatching success (Dehnel and Kong, 1979; Watt and Aiken, 2003). Additionally, with generation times shortening, mismatches between

hatch timing and seasonal food availability may occur (Rivkin, 1991; Both *et al*., 2006) compromising hatchling's survival.

Lastly, metabolism may be regarded as one of the most markedly temperature-dependent physiological functions in a given organism (Frederich and Pörtner, 2000; Pörtner, 2001, 2002; Pörtner *et al*. 2004; Pörtner *et al*. 2005; Pörtner and Knust, 2007). Beyond a certain temperature, "pejus temperature", oxygen delivery cannot continuously rise to cover higher metabolic demands (Frederich and Pörtner, 2000; Pörtner, 2001, 2002; Pörtner *et al*. 2004, 2005), which will influence an organism's ability to feed, growth and reproduce (Pörtner and Knust, 2007). Studies of oxygen consumption rates in adult nudibranchs, showed that beyond their thermal limits, the individuals were energetically stressed (Potts, 1983; Smith and Sebens, 1983; Havenhand and Todd, 1988a).

Organisms under situations of environmental stress such as, extremes of temperature or starvation may reduce the scope for activity (Newell, 1973), activating a metabolic suppression response (Grieshaber *et al*., 1994). This way, they can minimize the use of metabolic reserves during periods of stress and allow the allocation of energy to increase their metabolic rate and activity when suitable environmental conditions prevail (Newell, 1973). The correlation between temperature and oxygen consumption rates has been proved especially important during nudibranch's early-life stages, since their performance and survival strongly influences population dynamics (Moran and Woods; 2007; Woods and Moran, 2008).

The aim of this study was to scrutinize, for the first time, the impact of a near-future global warming scenario on the physiological ecology of a specialized-coral feeding nudibranch, *Armina maculata*. Adult breeding pairs and egg masses were incubated under four different thermal conditions, which reflected: i) the present-day average spring (18ºC), early-summer (22ºC) and summer (24ºC) temperatures, and ii) the projected near-future warming (27ºC, +3ºC, IPCC 2007), a temperature that the species already experiences during heat wave events in Sado's estuary. In the present research, several parameters were evaluated in order to highlight the temperature effect on: i) adult feeding and survival ii) spawning frequency, iii) ontogenetic morphology; iv) embryonic development time and survival, and v) adult and embryo's metabolic rates and thermal sensitivity (Q_{10} values).

2. MATERIAL AND METHODS

2.1. Sampling and adult's maintenance

Adults of the nudibranch *Armina maculata* (Rafinesque, 1814) were collected in Sado's estuary (West coast of Portugal) (Fig.1), in the summer period of 2011. After collection, nudibranchs were immediately transferred to the aquaculture facilities in Laboratório Marítimo da Guia, Cascais.

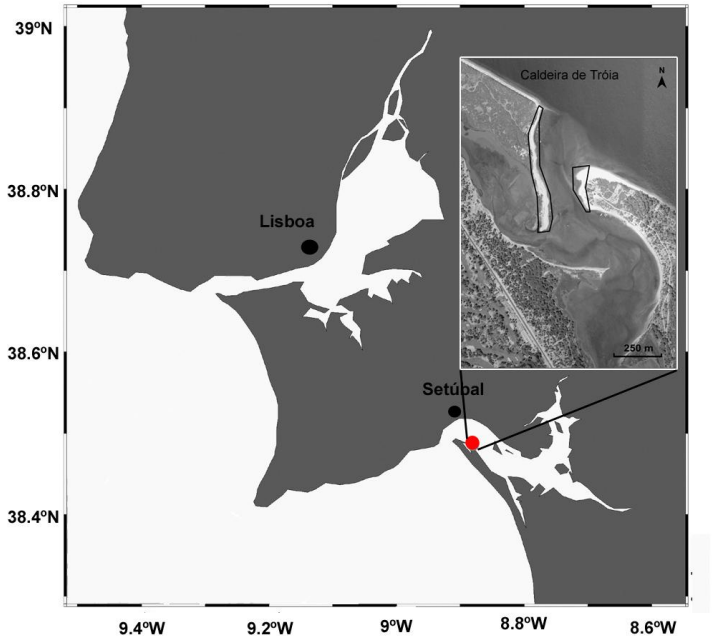

Figure 1 - Map of the sampling area, the mouth of Sado's estuary (red circle), Portugal. The polygons indicate the extension of the sampling area (~100,000 m^2)

After 48h of acclimation, *A. maculata* individuals were placed in pairs, three at each experimental condition, to allow copulation trials. Maintenance was carried out in four recirculating systems, each containing 25 separated glass aquariums (volume of 54L) and a collective sump of 270L. The closed systems were filled with UV-sterilized and filtered (series 20, 10, 5 and 1 µm) seawater and tanks were placed on with a photoperiod of 14-h light:10-h dark cycle. Water quality was ensured using wet-dry filters (bioballs), protein skimmers (Schuran, Jülich, Germany) and 30W UV-sterilizers (TMC, Chorleywood, UK). Ammonia and nitrite were monitored regularly and kept below detectable levels. Salinity was kept at 34.0± 1.0 and pH was maintained at 8.1±0.1.Temperature was regulated via Heilea chillers (Guangdong, China).

Experimental temperatures were chosen to reflect: i) the present-day average spring (18ºC), early-summer (22ºC) and summer (24ºC) temperatures, and ii) the projected near-future warming (27ºC, +3ºC, IPCC 2007), a temperature that the species already experiences during heat wave events in Sado's estuary.

Aquariums were covered with a sandy substrate to allow egg masses settlement, since this species show an exquisite burying egg-laying behavior. Nudibrachs were fed with the pennatulcean octocoral (sea pen) *Veretillum cynomorium* colonies *ad libitum*.

2.2. Adult survival and feeding assays

In all experimental conditions, mortality (during the incubation period) was registered, for each breeding pair and temperature, to calculate: i) adult survival in weeks and ii) adult survival rates.

Feeding assays were performed to verify the preference of *A. maculata* in its dietary prey, the octocoral *V. cynomorium*, and to analyse the influence of warming in food intake. Food intake was defined as the number of colonies of *V. cynomorium* eaten by individual per week in all experimental conditions. Five experimental trials were conducted for each of the four thermal treatment (with three breeding pairs per temperature).

Regarding prey species preference, experiments were conducted only at 18ºC (control conditions) in order to avoid the effect of temperature in individual tanks. After submitting the nudibranchs (n=5) to a starvation period of 48h, one small piece of different prey organisms was placed in each tank: shrimp (*Palaemon elegans*), mussel (*Mytilus galloprovincialis*), fish (*Sardina pilchardus*) and *V. cynomorium* colonies. After 24h, tanks were inspected to register which preys were eaten.

2.3. Spawning

For each breeding pair, at each thermal treatment, the number of egg masses laid was daily registered to quantify the spawning frequency, i.e. number of egg masses per week (during seventeen weeks of incubation). The weekly spawning frequency was calculated

as the mean value of the total number of egg masses laid by the three separate breeding pairs placed at different thermal scenarios.

2.4. Egg collection and incubation

After laying, all spawn masses were excised and removed from the substrate. In order to minimize handling, egg masses (n=5) from each tank were carefully cleaved in four equal parts, individually photographed, identified and placed back in small floating containers (incubators) at the different thermal scenarios (18, 22, 24, 27ºC).

2.5. Embryonic morphology, development and survival

Observations and photographs on each egg mass were carried out immediately after laying, 12h after and then with a daily basis, using a Leica S6D dissecting microscope with a phototube attachment and a Canon EOS 550D digital camera. In each observation, developmental stage was identified and the following measurements were made for early and late stages: i) capsule length (longest diameter) and width (perpendicular to longest diameter), ii) number of embryos per capsule, iii) embryo diameter and iv) veliger length. For each trial, thirty measurements were made for all the parameters, in the early and late stages of ontogeny. Capsule and embryo measurements were used to determine the respective volumes, using the following formulas: i) capsule volume - assuming a prolate spheroid shape: $V = 4/3\pi LW^2$; ii) embryo volume, assuming a spheroid shape: $V = 4/3(R^3)$, [(V = volume, W = width, L = length, R = radius)]. The percentage of embryo survival at each temperature was calculated by the number of embryos alive per capsule in the early and late stages. Egg mass viability was defined as the percentage of egg masses in which more than 90% of the eggs successfully hatched as veliger larvae. All measurements were performed using the software ImageJ 1.45.

2.6. Oxygen consumption rates and thermal sensitivity

2.6.1. *Adults*

Nudibranchs were placed in an intermittent flow-through respirometry set-up (Loligo Systems, Denmark; see Rosa and Seibel, 2008, 2010) containing filtered (0.2 µm) seawater mixed with antibiotics (50 mg L^{-1} streptomycin) to avoid bacterial oxygen consumption. Respiration chambers were immersed in a large thermostatted (Lauda, Lauda-Königshofen, Germany) water bath to control temperature. Seawater was pumped at a constant flow rate (average 120 mL min^{-1}) from a water-jacketed, gas-equilibration column through the respirometers. The water in the column was bubbled continuously to maintain incoming water at high (normoxia, 21% O_2). Oxygen concentrations were recorded with two Clarke-type O_2 electrodes connected to a 928 Oxygen Interface (Strathkelvin Instruments, Scotland). The system was calibrated using air- and nitrogen-saturated seawater. During, before and after each trial, the experimental setup was checked for electrode drift and microbial oxygen consumption. The duration of respiratory runs varied from 5 to 16 h. For each temperature, thermal sensitivity (Q_{10}) was determined using the standard equation mentioned below.

2.6.2. *Embryos*

Oxygen consumption measurements (routine metabolic rates) were determined according to Rosa *et al.,* (2009, 2012) and Pimentel *et al.,* (2012). Egg masses were incubated in sealed water-jacketed respirometry chambers (RC300 Respiration cell, Strathkelvin, North Lanarkshire, Scotland) containing filtered seawater (0.2 µm) with antibiotics (50 mg L^{-1} streptomycin) to avoid bacterial respiration. Water volumes were adjusted in relation to animal mass (up to 4 mL) in order to minimize stress and allowing spontaneous and routine activity rates of the embryos. Bacterial controls were conducted to check possible bacterial respiratory activity. Respiration chambers were placed in water baths (Lauda, Lauda-Königshofen, Germany) to control temperature. Oxygen concentrations were recorded with Clarke-type O_2 electrodes connected to a multi-channel oxygen interface (Strathkelvin, North Lanarkshire, Scotland). The duration of respiratory runs varied from 3 to 6h.

For each temperature, thermal sensitivity (Q_{10}) was determined using the standard equation:

$$Q_{10} = [R(T_2) / R(T_1)]^{[10 / (T2 - T1)]},$$

where $R(T_2)$ and $R(T_1)$ represent the oxygen consumption rates at temperatures T_2 and T_1, respectively.

2.7. Statistical analysis

One-way ANOVA was used to evaluate the effect of temperature on metabolism rates in adults and embryos, veliger's length, adult survival, total number of egg masses, spawning frequency, development time independently for all five embryonic stages and food intake. Additionally, two-way ANOVAs were conducted to detect significant differences in number of embryos per capsule and capsule and embryo volumes between temperature and developmental stage (early and late stages). Previously, normality and homogeneity of variances were verified by Kolmogorov – Smirnov and Bartlett tests, respectively. Subsequently, post-hoc tests (Tukey HSD and unequal N HSD) were performed. All statistical analysis were performed for a significance level of 0.05, using Statistica 10.0 software (StatSoft Inc., Tulsa, USA).

3. RESULTS

3.1. Adult survival

Ocean warming significantly impacted adult survival during the experimental trials (Fig.2A, B, F= 6.2, p= 0.004). In fact, 33% of the adults incubated under the projected near-future warming scenario died at the 3rd week, and the remaining individuals died until the 7th week of incubation (Fig.2A). Thus, the mean adult survival rate at such thermal condition was 5 weeks (Fig. 2B). At summer temperature (24ºC), 17% of the individuals died at the 4th week of incubation, though 67% survived throughout the study (Fig.2A,B). In contrast, the percentage of survival was significantly greater under the present-day spring and summer scenarios, both with 83% of survival until the end of the experiment.

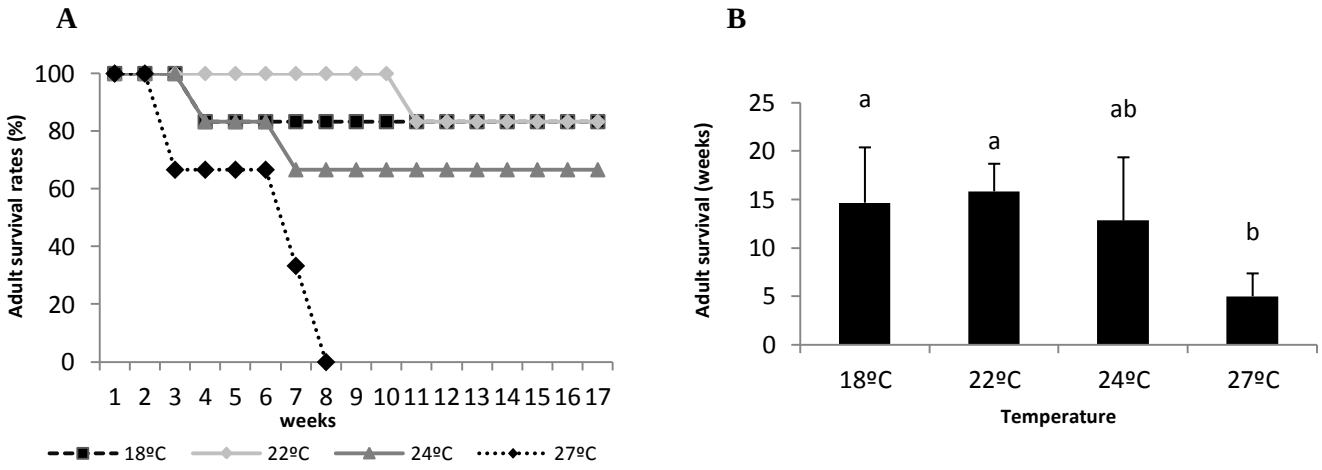

Figure 2 - *A. maculata* adult survival rates (A) under four different thermal scenarios during the first seventeen weeks of incubation (B), and in total number of weeks. Values are means ± SD. Different letters represent significant differences

3.2. Adult feeding assays

As expected, prey preference trials proved that *A. maculata* fed exclusively on the octocoral *V. cynomorium* (Fig. 3B), since there were no indications of predation on others available preys placed on the incubation tanks.

One of the most striking impacts of the future warming on *A. maculata's* ecology, was the significant inhibition of food intake with increasing temperature (Fig.3A, F= 18.9, p< 0.001). Higher food intake, was verified at the spring temperature (18ºC), with nudibranchs eating 1.19 ± 0.39 *V. cynomorium* colonies per week. The number of prey eaten per week decreased with temperature, and under the more extreme warming scenario (27ºC), nudibranchs stopped the feeding activity (Fig.3A).

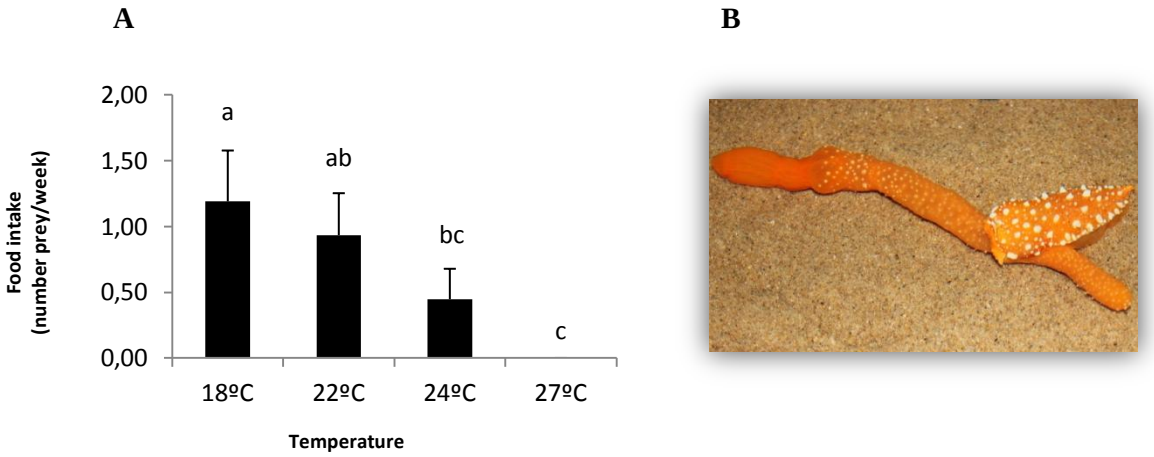

Figure 3 - Effect of temperature in *A. maculata's* food intake: number of prey items (*Veretillum cynomorium*) eaten by individual per week at 18, 22, 24 and 27ºC (A), and adult *A. maculata* feeding on the octocoral *Veretillum cynomorium* (B). Values are means of five measurements ± SD. Different letters represent significant differences

3.3. Spawning

In the present study, *A. maculata* breeding pairs showed frequent copulation events (Fig.4A) followed by oviposition (Fig.4B).

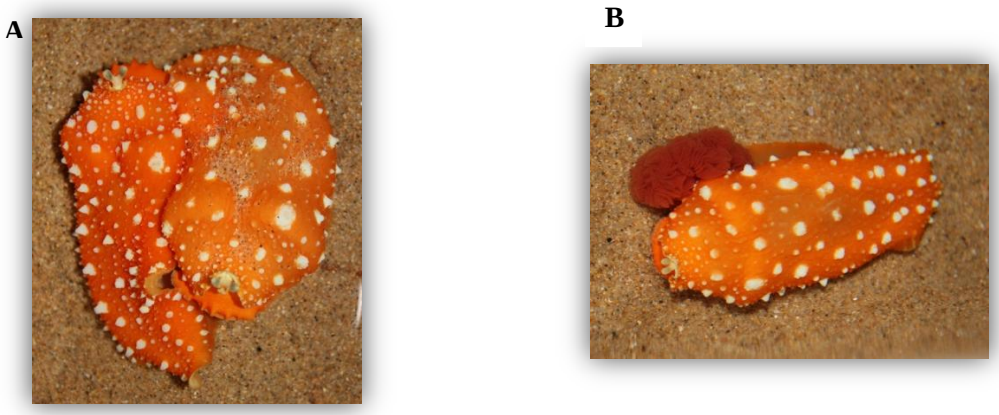

Figure 4 – A) *A.maculata* adult breeding pair copulating (~5-13cm individual l length), and B) adult laying an egg mass.

The spawning frequency (Fig.5B) was obtained for the first seventeen weeks of incubation, and results revealed a significant correlation between temperature and the number of egg masses laid per week (Fig.5C, F= 21.8, p<0.001) and total number of egg masses spawned until the end of the experiment (Fig.5D, F= 9.5, p= 0.005). Breeding pairs exposed to early-summer temperature (22ºC) spawned continuously during all experimental period (Fig.5C, 1.1 ± 0.9 egg masses per week), and a total of ~18 ± 6.1 egg masses were laid (Fig.5D). Regarding summer temperature conditions (24ºC), egg masses were observed until the 14th week however, a decrease in spawning frequency can be noticed (Fig.5B,C, ~0.7 ± 0.7 egg masses per week), comprising a total of ~10.3 ± 5.1 egg masses laid (Fig.5D).

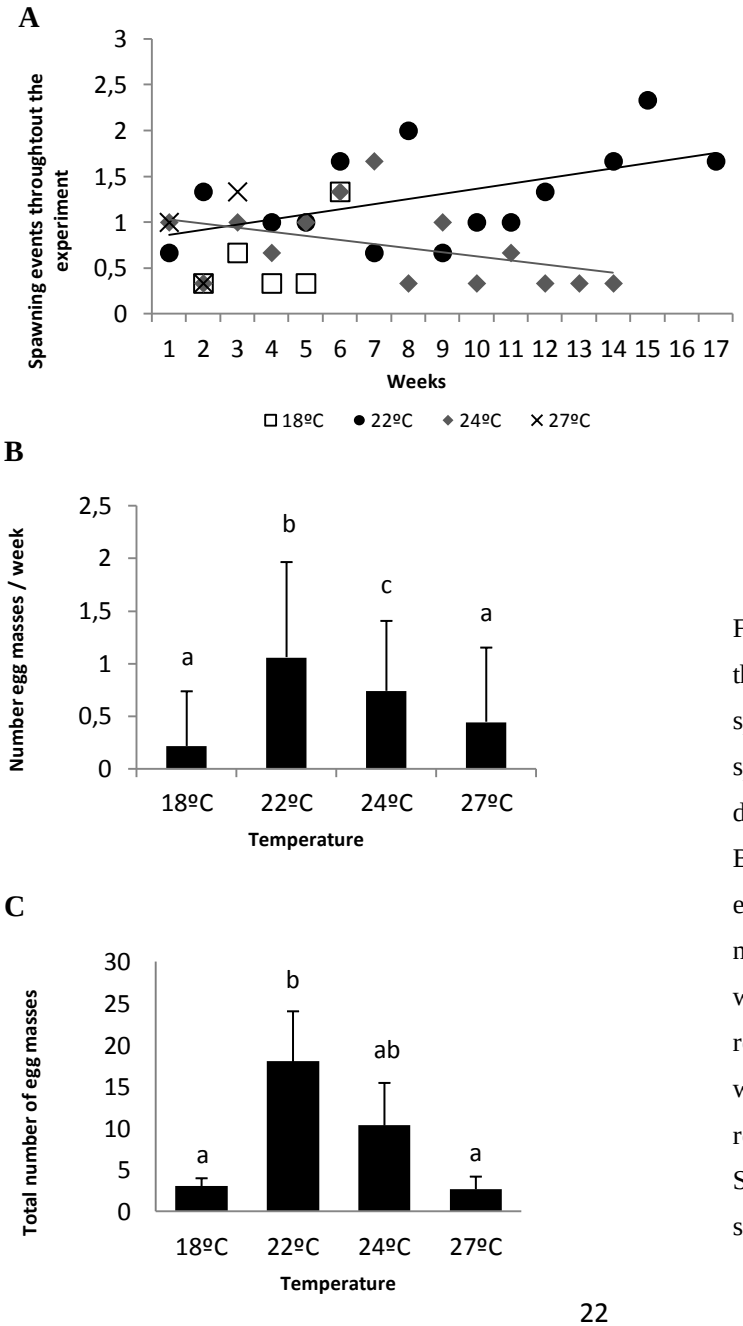

Figure 5 – The effect of four different thermal scenarios in *A. maculata's* spawning, namely: A) ,mean number of spawning frequency events per week during seventeen weeks of incubation, B)(B),spawning frequency (number of egg masses per week), C(C), and total number of egg masses during seventeen weeks of incubation (per each reproductive pair) (D), during seventeen weeks of incubation per each reproductive pair. Values are mean ± SD. Different letters represent significant differences.

After that period the breeding pairs did not show any reproductive behaviour and consequently egg masses were not found in the rearing tanks. On the other hand, spawning at spring temperature (18ºC) was only observed on the first six weeks with lower egg masses per week (Fig.5C, 0.2 ± 0.5 egg masses per week) comparing with all the other thermal treatments. Finally, the breeding pairs subjected to the projected near-future warming scenario (27ºC), only spawned during the first three weeks of incubation (Fig.5B), followed by post-spawning death of individuals, laying a total of ~2.7 ± 1.5 egg masses (Fig.5D).

3.4. Ontogenetic development

3.4.1. *Egg masses and embryos*

A. maculata nudibranchs laid coiled gelatinous egg masses, attached to the substratum. Each egg mass was covered by a thick transparent gelatinous matrix and contained several thousand embryos (Fig.6A). Embryos had a pink appearance and were contained in ellipsoid-shaped capsules that were densely packed within the spawn mass (Fig.6B).

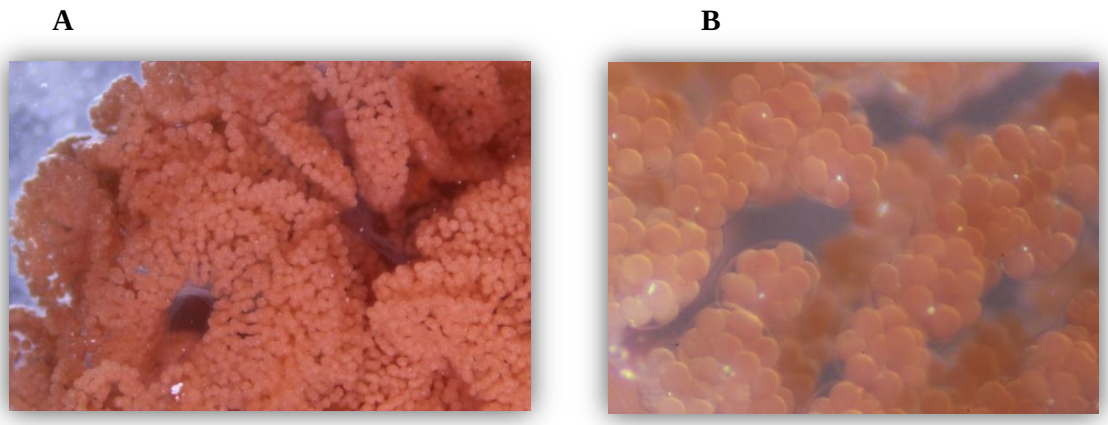

Figure 6 – Egg mass of an *A.maculata* nudibranch (A), and a detail of an egg mass, with capsules (77 ± 7.9 µm^3 mean volume) containing embryos (0.34 ± 0.03 µm^3 mean volume) (B) appearance during early-stages.

Temperature significantly affected capsule and embryo's volume during development (Fig.7A,B). Morphological measurements showed that egg masses incubated at early-

summer temperature (22ºC) had no significant changes in its capsules volume during embryogenesis, contrary to all other thermal treatments that showed a decrease in capsule volume of approximately 60% (Fig.7A, two-way ANOVA, $p<0.001$). Moreover, embryo's volume was significantly affected by temperature between early and late stages (Fig.7B, two-way ANOVA, $p<0.001$). Although there were no significant differences in embryo's volume during early stages between all thermal treatments (~0.34 ± 0.03 μm^3), at both spring (18ºC) and summer temperatures (24ºC), there was a significant increase of 16% and 42% during embryogenesis. Although not significant ($p<0.05$), under a future warming scenario embryo's volume increased about 23% during development (Fig.7B).

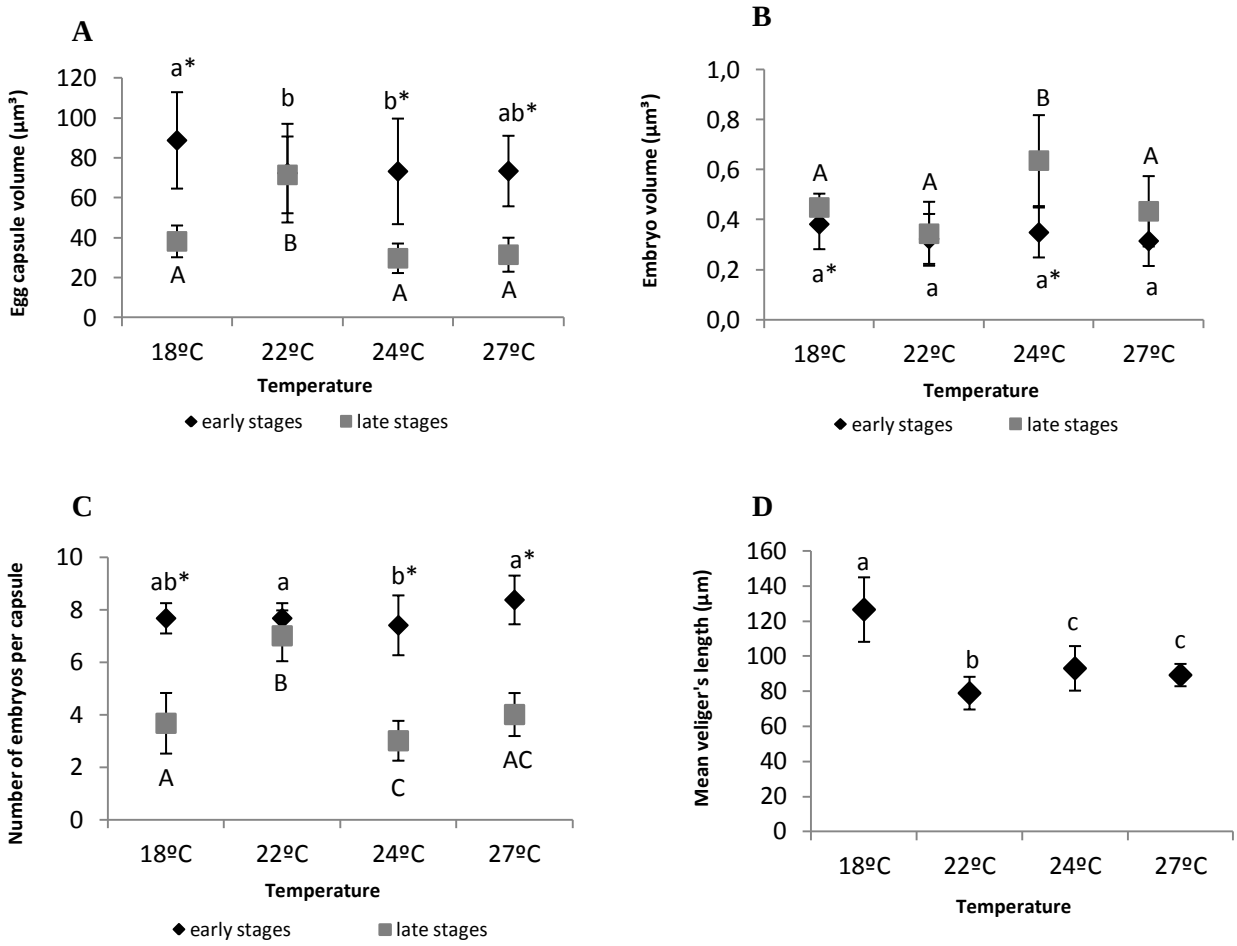

Figure 7 – Effect of warming in the early ontogeny of *A. maculata*, namely: capsule volume (μm^3) (A), embryo volume (μm^3) (B), number of embryos per capsule (C), veliger's length (µm) (D) at four different temperature scenarios. Values are mean ± SD. Different letters (capital letters for late stages; small letters for early stages) and asterisk represent significant differences between temperatures and developmental stages, respectively.

Additionally, the mean number of embryos per capsule was significantly reduced at all thermal treatments, except at the early-summer scenario (22ºC), where the number of embryos per capsule at late stages was (~7 ± 0.97) (Fig.7C, two-way ANOVA, $p<0.001$). In the beginning of the development, the mean number of embryos per capsule was ~8 for all temperatures, but decreased up to ~4 at the late stages under the spring (18ºC), summer (24ºC) and warming (27ºC) scenarios.

Lastly, veliger hatchlings length revealed a significant influence of temperature (Fig.7D, $F = 77.6$, $p<0.001$) with the largest size, 126.3 ± 18.4 µm, at spring temperature (18ºC) and the shortest, 79 ± 9.3 µm, at early-summer temperature (22ºC).

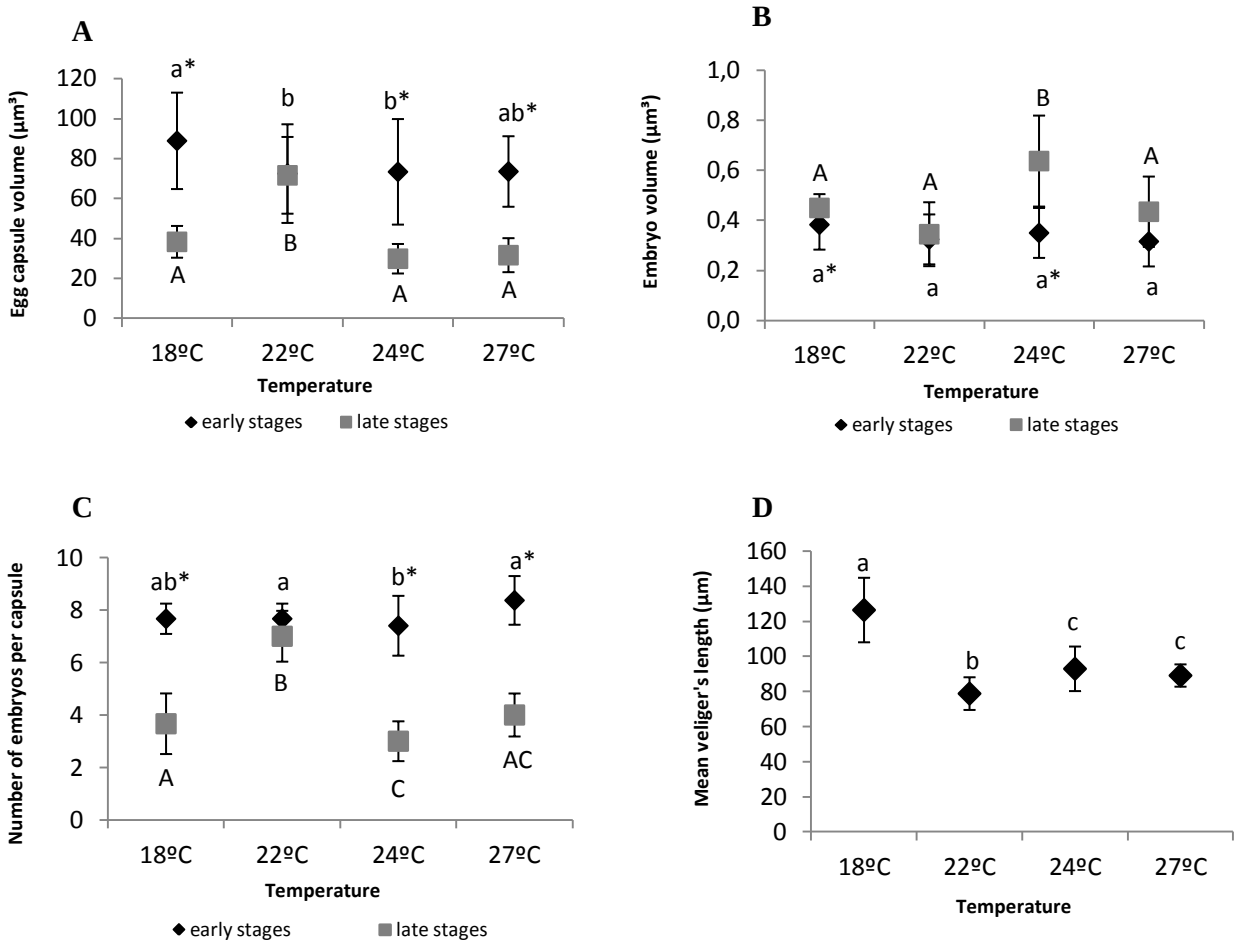

Figure 7 – Effect of warming in the early (blastula and gastrula) and late (trocophore and active veliger) embryonic stagesontogeny of *A. maculata*, namely: capsule volume (µm^3) (A), embryo volume (µm^3) (B), number of embryos per capsule (C), veliger's length (µm) (D) at four different temperature scenarios. Values are mean ± SD. Different letters (capital letters for late stages; small letters for early stages) and asterisk represent significant differences between temperatures and developmental stages, respectively.

3.4.2. *Embryo survival*

Embryo survival rates were much greater at early-summer scenario (22ºC), decreasing up to 45% under both present-day spring (18ºC) and summer (24ºC) conditions, as well as under the projected near-future warming (27ºC) (Fig.8).

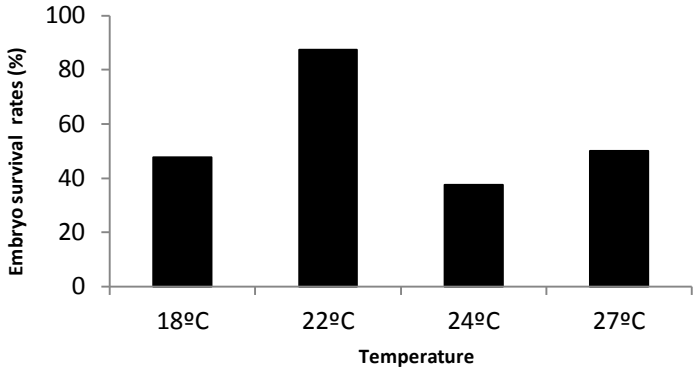

Figure 8 - Percentage of embryo survival at four different temperature scenarios.

3.5. Embryonic development time and success

Five developmental stages on *A. maculata* were identified: blastula, gastrula, trocophore, active veliger and newly-hatched veliger (Fig.9A). As expected, the duration of the embryonic stages was significantly shortened with increasing temperature, especially in the beginning (Fig. 9A, blastula stage, $F= 4.1$, $p = 0.02$ and gastrula stage, $F= 4.3$, $p = 0.02$) and in the end of the development (Fig.9A, newly-hatched veliger stage, $F = 7.6$, $p<0.001$). Although there was a noteworthy temperature-developmental stage interaction, there was no significant effect ($p>0.005$) in both trocophore and active veliger's stages as shown in Figure 9. The mean cumulative time from fertilization to hatching under present-day scenarios was 7.6 ± 0.5 days at spring temperature (18ºC), 6.2 ± 0.4 days at early-summer (22ºC) and 6.2 ± 0.8 days at summer temperature (24ºC). In a future warming scenario (27ºC), development time significantly decreased, with a mean of 5 days from spawning to hatching (Fig. 9A, $F = 7.6$, $p<0.001$).

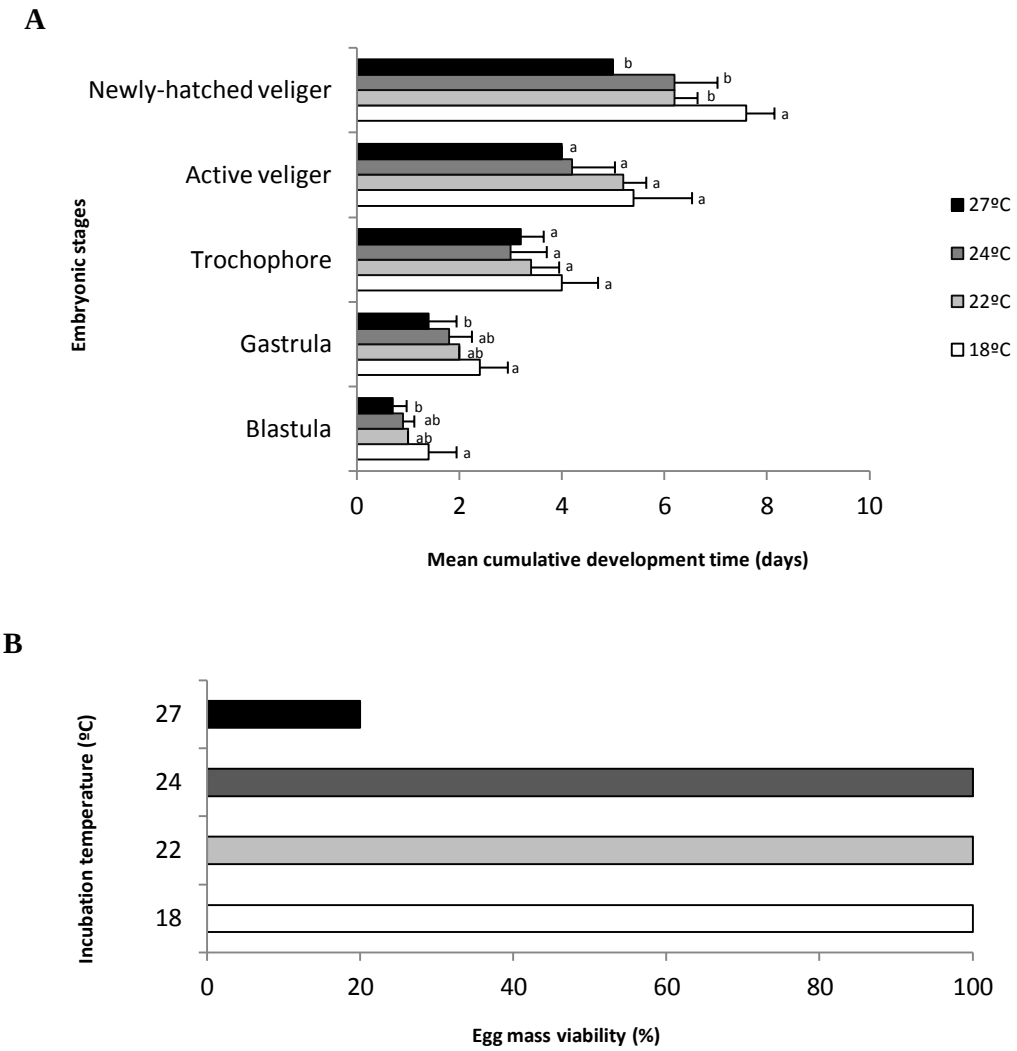

Figure 9 – Mean cumulative development time (days ± SD) for of *A.*the different early ontogenetic stages of *A. maculata* at 18, 22, 24 and 27ºC; different letters represent significant differences (A) and the respective percentage of egg mass viability (B).

Furthermore, the projected near-future warming scenario elicited a significant negative effect on embryonic success, with egg mass viability decreasing 80% (Fig.9B). At this temperature, four of the five egg masses observed developed a very different pattern from that seen at lower temperatures, as described above. Regular development on the highest temperature never proceeded after the gastrula stage and no veliger's ever hatched. Regarding color and morphological features, the entire egg mass appeared yellowish and capsules were white-opaque (Fig.10A). Embryos exhibited abnormal cell divisions that produced either uneven cleavage or no cleavage at all, indicating that they were either unfertilized or dead (Fig.10B).

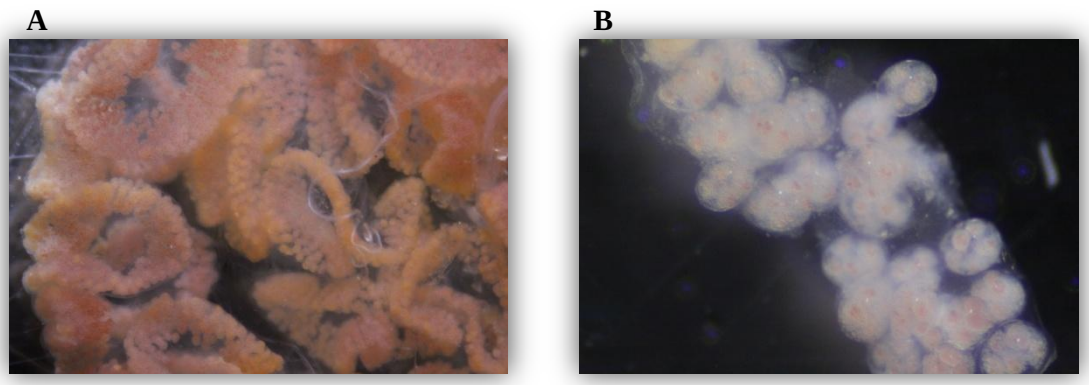

Figure 10 – Effect of warming in *A.maculata*'s egg masses incubated at 27ºC: visible deleterious effects in the whole egg mass appearance (A) and, visible deleterious effects in capsules (31.4 ± 8.5 μm^3 mean volume) and embryos (0.43 ± 0.1 μm^3 mean volume) (B).

3.6. Oxygen consumption rates and thermal sensitivity

Mass-specific oxygen consumption rates (OCR) of adults ranged from 0.13 µmol O_2 $h^{-1}g^{-1}$ at spring temperature (18ºC) to 0.22 µmol O_2 $h^{-1}g^{-1}$ at the warming scenario (Fig.11A). The Q_{10} values decreased with the increase of temperature, ranging around 2 at spring temperature (18ºC) and around 1.5 (indicative of active metabolic suppression) at the warming condition (27ºC) (Fig.11B).

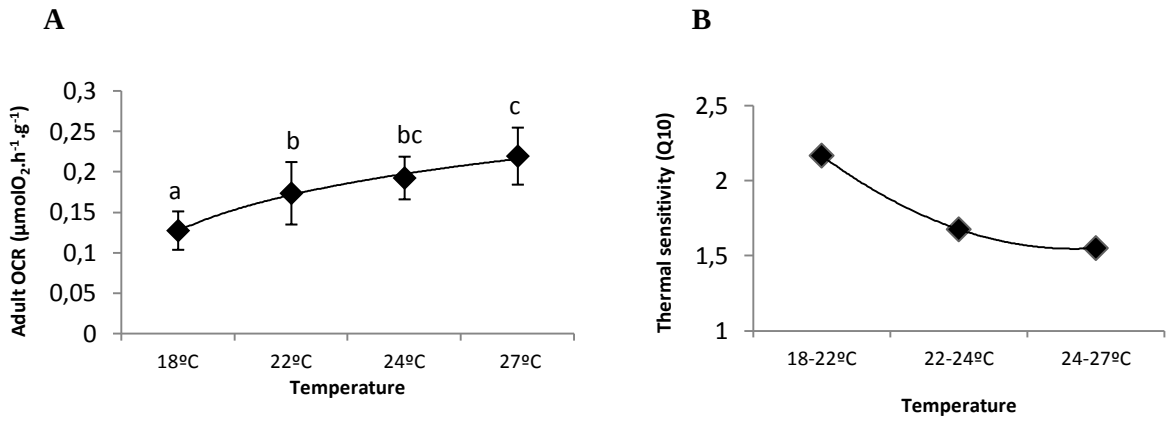

Figure 11– Metabolic rates (MO_2, µmol O_2 g^{-1} h^{-1}) of *A. maculata* adults (A), and thermal sensitivity (Q_{10}) of *A. maculata* adults, at four different temperatures: the present-day average spring (18ºC), early-summer (22ºC) and summer (24ºC) temperatures, and the projected near future warming (27ºC) in Sado estuary. Values are means of twelve measurements ± SD. Different letters represent significant differences. Q_{10} values between 2 and 3 indicate active metabolic regulation; Q_{10} values inferior to 1.5 suggest active metabolic suppression

Metabolic rate increment with temperature was higher for embryos than for adults. In fact, embryo's OCR of *A.* ranged from 0.009 µmol O_2 h^{-1} $embryo^{-1}$ at spring temperature (18ºC) to 0.02 µmol O_2 $h^{-1}embryo^{-1}$ at the projected near future warming condition (Fig.12A, F = 14.5, p< 0.001). Embryonic stages displayed Q_{10} values ranging mainly between 2.5 and 3.5, however, under the future warming scenario (27ºC), Q_{10} values slightly decreased to 1.9 (Fig.12B). At normal operating temperatures, metabolic demand for oxygen increases with temperature with Q_{10} around 2-3.

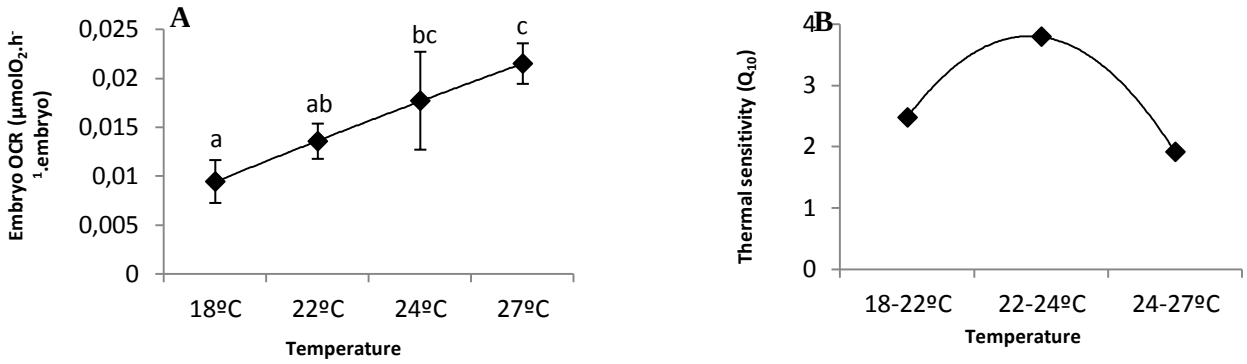

Figure 12 - Metabolic rates (MO_2, µmol O_2 g^{-1} h^{-1}) of *A. maculata* embryos (A), and thermal sensitivity (Q_{10}) of *A. maculata* embryos, under four different thermal scenarios. Values are means of twelve measurements ± SD. Different letters represent significant differences. Q_{10} values between 2 and 3 indicate active metabolic regulation; Q_{10} values inferior to 1.5 suggest active metabolic suppression.

4. DISCUSSION

4.1. Adult survival, feeding and spawning

Adult survival, feeding and spawning results may not be analyzed independently, since all these parameters correlate and contribute to a species reproductive fitness (Todd, 1979; Smith and Sebens, 1983; Thompson, 1985; Havenhand and Todd 1988b). Therefore we could verified that, as adult survival and food intake rates declined with temperature increment, so did the *A.maculata*'s reproductive effort (Fig.2-4).

It is known, that adult nudibranchs allocate most of their energetic reserves to ensure reproductive success (Havenhand and Todd, 1988b). The search for an hermaphroditic partner, copulation, egg mass production and spawning activities require a large energy investment (Havenhand and Todd, 1988b), and many adult nudibranchs deplete all their energetic resources during the spawning period, leading inevitably to their death (Todd, 1979; Smith and Sebens, 1983; Havenhand and Todd 1988b; Jones *et al.*, 1996). In our study, adult nudibranchs incubated under future warming conditions (27ºC) never displayed any feeding activity and died after seven weeks of incubation (Fig.3A).

Possibly, under a 27ºC scenario, the organism dispended most of its energy reserves trying to keep pace with increased metabolic demands (see Fig. 11A) (Pörtner and Knust, 2007), but after a period of time exposed to a high temperature, the organism physiological response triggered a shutdown-chain reaction (i.e. enzymatic systems failures, protein denaturalization, respiratory stress and membrane damages) coupled with active metabolic suppression (see Fig. 11A) and the individual ceased feeding, reproduction, locomotion and finally died (Wang and Overgaard, 2007; Katersky and Carter, 2007).

Regarding feeding preferences trials, our data provide evidence that *A.maculata* preferred feed on the octocoral *Veretillum cynomorium*, even when offered alternative prey items, showing the expected stenophagous behavior. Indeed, many nudibranchs display a species-specific, predator-prey association (Harris, 1973; Todd, 1981, 1983; Smith and Sebens, 1983; Carrol and Kempf, 1990; Wagner *et al*., 2009). As specialized predators, stenophagous species have intimate ecological relationships with their prey (Wagner *et al*., 2009), however this feeding dependence might impact *A.maculata* survival under a warming scenario, since the local extinction of the preferred prey could

cause a reduction in the feeding activity, providing lower energy to support the expected higher metabolic rate (Smith and Sebens, 1983) leading eventually local extinction.

Many marine organisms are known to have a window of optimal temperature for spawning (Chia, 1973; Strathmann, 1987; Stanwell-Smith and Peck, 1998; Carmona-Osalde *et al*., 2004; Byrne, 2011). Smith and Sebens (1983) proposed that the nudibranch *Onchidoris aspera* spawning events were limited to a time period when sea-water temperatures ranged from -1.0 to 4.0ºC and adult individuals were only reproductively active during that season, to insure offspring survival. In fact, it was been stated that the spawning season duration, frequency and viability of egg masses were directly affected by temperature in nudibranchs (Thompson, 1985). This reproductive optimal thermal range, as well as differences in fecundity, reflect an adaptation to the habitat environmental conditions thus, differ even among closely related species from different biogeographical locations (Llorda, 2002).

There are no studies describing *A.maculata*'s reproductive ecology, however, our observations in adult's spawning, also suggested the existence of an optimal breeding temperature for this species (22ºC), where the mean number of egg masses and oviposition's frequency increased throughout the experimental trial (Fig.5A,B). On the other hand, the adult breeding pairs incubated at spring (18ºC) and summer (24ºC) temperatures, showed a shorter spawning period (six and fourteen weeks, respectively) and a smaller amount of egg masses laid per week (Fig.5B). Considering these results, we may suggest that this *A.maculata* population starts spawning during the early-summer, when sea-temperatures range ~22ºC. Yet, as already described by other authors (Capo *et al*., 2002; Plaut *et al*., 1995) in the presence of hermaphroditic conspecifics and food availability, they can be reproductively active outside their optimal thermal window although with a poorer reproductive fitness.

Under the future warming scenario (27ºC) spawning events were scarce and lasted for only three weeks, revealing that higher temperatures have a significant negative impact in this species reproduction (Fig.5A-C).

To overcome this future warming scenario, we could argue that *A.maculata* may undertake short migrations as an evolutionary adaptive response, as reported for other nudibranch species (Crozier, 1917; Pelseneer, 1922; Claverie and Kamenos, 2008), to find cooler environments, where temperatures range within its thermal limits.

Additionally, *A.maculata* nudibranchs may take advantage of their typical burying behavior (Rafinesque, 1814), spending more time covered by the substrate, where temperatures should be lower.

4.2. Embryonic morphology, development and success

Although there are no studies concerning *A. maculata*'s reproductive traits (egg size, spawn mass size, embryonic development rate, larval-type and hatching success) it is known that intraspecific variation between conspecifics is frequent among nudibranchs (Jones et al., 1996). For instants, the number of embryos produced per spawning depends on parental size, and can range from 3 to 272 x 10^6, depending on the species (Switzer-Dunlap and Hadfield, 1979; 1984). Embryo and capsule volume measurements, are frequently used to evaluate parental nutritional state (Jones *et al.*, 1996), development energetic costs (Jones *et al.*, 1996) and permeability to oxygen diffusion (Woods and Moran, 2008).

As we expected, capsules and embryos underwent significant volume changes between early and late stages at all temperature conditions (18, 24 and 27ºC), except at the early-summer temperature (22ºC) (Fig.7A,B). Capsules were smaller at late stages, while embryos were larger. The underlying causes of these results are unknown, but we suggest that it must be related to embryo's survival during development and embryo packing density. Considering the latter, according to the theoretical maximum packing of jammed disordered ellipsoids (see Donev *et al.*, 2004), embryos cannot occur at higher densities than ~74% inside the capsules, however, if there is available space inside the capsule as a consequence of embryo mortality, the remaining embryos will increase volume fulfilling that space. Indeed, capsule volume places a limit on embryo density that Woods and Moran (2008) considered of great importance in concern to oxygen-supply dynamics during embryonic respiration (discussed below).

The effect of temperature in embryo survival has been described for several invertebrate marine organisms (Stanwell-Smith, 1998; Przeslawski, 2004; Pimentel *et al.*, 2012; Rosa *et al.*, 2012) and for all cases, embryo viability declined with increasing temperature. At 22ºC we observed the highest percentage of embryo's survival (~88%), on the other hand, at the other thermal conditions (18, 24 and 27ºC) embryo's survival

decreased up to ~45% (Fig.8). The stressful abiotic conditions inside the eggs, such as higher metabolic rates (see also Moran and Woods, 2007; Woods and Moran 2008), high levels of hypoxia (Moran and Woods, 2007; Woods and Moran 2008) and embryo low energetic reserves, due to parental deficient nutritional state (Jones *et al*., 1996; Llorda, 2002), may explain the results achieved for higher temperatures (24 and 27ºC). To justify the high embryo mortality rates at 18ºC, we may argue that this temperature is below *A.maculata*'s reproductive thermal window.

In nudibranchs, egg size is related to larval development mode (Jones *et al.*, 1996) and, on the other hand, hatchling size is positively correlated with hatchling organic content (Moran and Emlet, 2001) that depends directly on the parental energy reserves (Jones *et al*., 1996; Llorda, 2002) and so, may be used as an indicator of maternal investment (Moran and Emlet, 2001). Our findings also showed a correlation between egg sizes and veliger's length that varied significantly between experimental temperatures (Fig.7A,D).

Most marine invertebrates have a distinct larval phase in their early life histories and can be divided into species whose larvae feed in the plankton (planktothophic) and species whose larvae can develop and metamorphose without feeding (lecithotrophic) (Strathmann, 1985; Pechenik, 1987). Therefore, the effects of warming in the early ontogeny will also greatly depend on the type of larval developmental modes (Woods and Moran, 2008; Byrne, 2011). The larval type of *A. maculata* was inferred to be planktotrophic trough egg size comparisons to other nudibranch species (as in Jones *et al*., 1996), such as *Onchidoris muricata* (Todd, 1987) and *Onchidoris aspera* (Smith and Sebens, 1983), and trough the observed high number of small sized eggs per egg mass, typical of a "r" strategy (Pianka, 1970), that favors higher number of offspring surviving natural mortality, instead of higher individual fitness (Boavida-Portugal *et al*., 2010). Since planktonic larvae strategy already comprises greater variability of individual offspring survivorship (Strathmann, 1985), under a warming scenario successful reproduction would be perhaps best achieved by restrained investment in a large number of temporally and spatially separated spawn masses (Havenhand and Todd 1988b).

The effect of temperature on development rates has been already stated for several marine invertebrate embryogenesis (Thompson, 1966, 1967; McMahon and Summers, 1971; Morse, 1971; Chia, 1973; Lalli and Conover, 1973; Williams, 1974; Kress, 1975;

Perron and Turner, 1977; Harris *et al*., 1980; Todd and Havenhand, 1985; Eyster, 1986; Strathmann, 1987; Farfan and Ramirez, 1988; Bouchaud, 1991; Boletzky, 1994; Steer *et al*., 2003; Przeslawski, 2004; Byrne, 2011; Pimentel *et al*., 2012; Rosa *et al*., 2012) as particularly for nudibranchs (Dehnel and Kong, 1979; Watt and Aiken, 2003).

Embryonic development time of *A. maculata* has never been described, however our results match the ones observed by Wagner *et al*. (2009) for *Phyllodesmium poindimiei* with a mean of six to seven days of development.

Here, we showed that embryonic development time was significantly shortened by temperature increment (Fig.9A). Although there was no significant temperature effect on developmental time in both trocophore and active veliger's stages, it was evident that, under higher temperatures, the extent of each embryonic stage will be shortened. This shortening in generation times will implicate a shift in the timing of release of veligers (Todd and Doyle, 1981; Byrne, 2011). Since the time period that new hatchlings can survive without food at higher temperatures is known to be very limited (Jones et al., 1996; Vidal et al. 2002), shifts in hatch timing can result in mismatches with seasonal food availability (Rivkin, 1991; Both et al., 2006) and comprise planktotrophic larvae survival under a future warming scenario (27ºC).

Furthermore, concerning an egg mass viability, our results showed a very low percentage (~20%) at 27ºC (Fig.9B) coupled with visible deleterious effects in egg masses (Fig.10A). Healthy egg masses were gelatinous and included inside a transparent matrix several thousand of transparent capsules containing the embryos, that conferred a pink appearance to the entire egg mass. But under the future warming condition (27ºC), the development did not proceed beyond the gastrula stage and the entire mass appeared yellowish, with some eggs exhibiting a white-opaque coloration. The embryos did not develop, indicating that they were either unfertilized or dead (Fig.10B). Similar results have been reported from Dehnel and Kong (1979) and Watt and Aiken (2003) for *Cadlina luteomarginata* and *Dendronotus frondosus*, respectively.

4.3. Oxygen consumption rates and thermal sensitivity

The effect of temperature in adult and embryo's nudibranch metabolism has been already focused in other studies (Potts, 1983; Smith and Sebens 1983; Havenhand and

Todd 1988a; Woods and Moran, 2008) and in all cases, oxygen consumption increases with temperature. In the present study, as expected, increased temperatures led to significant higher metabolic rates both in adults and embryos (Fig.11A).

For all organisms, temperature can increase to a point as long as cardiac and ventilatory adjustments can follow increase metabolic demands (Pörtner and Knust, 2007). Potts (1983) debated the great importance of gills and body epithelium in adult nudibranchs respiration, in overcoming the challenges of thermal stress. However, in this study we verify that *A.maculata* adult died after incubation under the future warming conditions (27ºC). In fact, it has been shown that beyond an organism thermal limit, anaerobic pathways, together with protein denaturation and permanent inactivation of enzymes, growth ceases and eventual death occurs (Wang and Overgaard, 2007; Katersky and Carter, 2007). This findings were corroborated with thermal sensitivity data, which revealed that *A. maculata* adults displayed Q_{10} values decreasing with temperature, showing values around 1.5 at warming (at 24º - 27ºC), which might be a consequence of active metabolic suppression (Thorp and Covich, 2001) (Fig.11B).

At all temperature scenarios, the enhancement in OCR was much steeper in embryos than in adults (Fig.12A). Early life stages such as eggs and hatchlings have been shown to be more vulnerable to environmental changes than adult organisms (Ishimatsu *et al*., 2008; Melzner *et al*., 2009; Byrne, 2011). The strong effect of temperature on oxygen consumption by embryos suggested that egg mass metabolism should be affected by even small changes in temperature (Woods and Moran, 2008). The underlying causes of this result might be related to: i) lack of physiological mechanisms to keep low metabolic rates inside eggs, as gills and body epithelium in adult nudibranchs respiration (see Potts, 1983) ii) high embryo packing density in eggs that contribute to accelerate oxygen deficiencies and might induce metabolic suppression (Cohen and Strathmann, 1996; Woods and Moran 2008), and iii) lack of physical protection by the egg mass structure that may increase embryo exposure to temperature changes (Todd, 1981; Woods and Moran, 2008).

In many ectotherms at normal operating temperature, metabolic demand for oxygen greatly increases with temperature (Q_{10} = 2-3) (Moran and Woods, 2007). Our results showed that embryos displayed Q_{10} values ranging around 2.5 – 3.5 for present-day temperatures (interval 18-22ºC), however, a pronounced drop above 24ºC brought Q_{10}

values to 1.9 was observed, which may suggest that oxygen deficiencies inside the eggs were aggravated by increasing temperature (Grieshaber *et al*., 1994) since those high temperatures are already outside embryos tolerance window (Fig.12B).

Susceptibility to warming may be the bottleneck for species persistence and ecological success in a changing ocean. Thus, compromised performance of early-developmental stages has deleterious consequences for adult populations and marine communities (Pimentel *et al*., 2012).

In the future, especially for adult *A.maculata*'s nudibranchs with high metabolic rates and low levels of metabolic reserves, feeding intake success would be crucial to organisms that require more food per unit body size. Under the projected near-future ocean warming, cease of feeding behavior, as observed, entails deleterious effects on this nudibranch's survival and growth.

5. FINAL REMARKS

The present findings showed that temperature plays a key role in delimiting *A.maculata*'s life cycle. Under the projected near-future warming scenario (+ 3ºC; 27ºC, IPCC) lower spawning frequency, higher adult and embryo mortality rates, as well as higher metabolic demands coupled to unsuccessful adult feeding intake, may compromise nudibranch adaptation success.

REFERENCES

Bhaud M, Cha JH, Duchêne JC, Nozais C (1995) Influence of temperature on the marine fauna: what can be expected from a climatic change. J Therm Biol 20: 91-104.

Boavida-Portugal J, Moreno A, Gordo L, Pereira J (2010) Environmentally adjusted reproductive strategies in females of the commercially exploited common squid *Loligo vulgaris*. Fish Res 106: 193–198.

Boletzky SV (1994) Embryonic development of cephalopods at low temperatures. Antar Sci 6(2): 139–142.

Both C, Bouwhuis S, Lessells CM, Visser ME (2006) Climate changeand population declines in a long-distance migratory bird. Nature 441, 81-83.

Bouchaud O (1991) Energy consumption of the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) during embryonic development, preliminary results. Bull Mar Sci 49(1–2): 333–340.

Byrne M (2011) Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. Ocean Mar Biol 49: 1–42.

Capo TR, Fieber LA, Stommes DL, Walsh PJ (2002) The effect of stocking density on growth rate and maturation time in laboratory-reared California sea hares. Contemp Topics Lab Animal Sci. 41, 18-23.

Carmona-Osalde C, Rodriguez-Serna M, Olvera-Novoa MA, Gutierrez-Yurrita PJ (2004) Gonadal development, spawning, growth and survival of the crayfish *Procambarus llamasi* at three different water temperatures. Aquaculture 232: 305–316.

Carroll DJ, Kempf SC (1990) Laboratory culture of the aeolid nudibranch *Berghia verrucicornis* (Mollusca, Opisthobranchia): some aspects of its development and life history. Biol. Bull. 179: 243-253.

Chia FS (1973) Oviposition, fecundity, and larval development of three sacoglossan opisthobranchs from the Northumberland Coast, England. The Veliger 13:319-325.

Claverie T, Kamenos NA (2008) Spawning aggregations and mass movements in subtidal *Onchidoris bilamellata* (Mollusca: Opistobranchia). J Mar Biol Ass United Kingdom 88 (1): 157:159.

CrozierWJ (1917) On a periodic shoreward migration of tropical nudibranchs. Amer Natur 51: 377-382.

Dehnel PA, Kong DC (1979) The effect of temperature on developmental rates in the nudibranch *Cadlina luteomarginata*. Canadian J Zoo 57: 1835-44.

Donelson JM, Munday PL, McCormick MI, Nilsson GE (2011) Acclimation to predicted ocean warming through developmental plasticity in a tropical reef fish. Global Change Biol 17: 1712–1719.

Donev A, Cisse I, Sachs D, Variano EA, Stillinger FH, Connelly R, Torquato S, Chaikin PM (2004). Improving the density of jammed disordered packings using ellipsoids. Science 303, 990-993.

Edwards M, Richardson AJ (2004) Impact of climate change on marine pelagic phenology and trophic mismatch. Nature 430: 881–884.

Eyster LS (1986) The embryonic capsules of nudibranch molluscs: literature review and new studies on albumen and capsule wall ultrastructure. Bull Am Malacol Soc 4:205-216.

Farfan BC, Ramirez LFB (1988) Spawning and ontogeny of *Bulla gouldiana* (Gastropoda: Opisthobranchia: Cephalaspidea). The Veliger 31:114-119.

Farkas T, Nevenzel JC (1981) Temperature acclimation in the crayfish: effects on phospholipid fatty acids. Lipids 16 (5), 341–346.

Frederich M, Pörtner HO (2000) Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, Maja squinado. Amer J Physiol 279:1531–1538.

Grieshaber MK, Hardewig I, Kreutzer U, Pörtner HO (1994) Physiological and metabolic responses to hypoxia in invertebrates. Rev Physiol Biochm Pharmacolg 125: 43–147.

Harris LG (1973) Nudibranch associations. Curr. Top. Comp. Pathobiol 2: 213-315.

Harris LG, Powers M, Ryan J (1980) Life history studies of the estuarine nudibranch *Tenellia fuscata* (Gould 1870). The Veliger 23:70-74.

Havenhand JN, Todd CD (1988a) Physiological ecology of *Adalaria proxima* (Alder et Hancock) and Onchidoris muricata (Müller) (Gastropoda : Nudibranchia). I. Feeding, growth and respiration. J Exp Biol Ecol 118: 151-172.

Havenhand JN, Todd CD (1988b) Physiological ecology of *Adalaria proxima* (Alder et Hancock) and *Onchidoris muricata* (Müller) (Gastropoda : Nudibranchia). II. Reproduction. J Exp Biol Ecol 118: 151-172.

Ishimatsu A, Hayashi M, Kikkawa T (2008) Fishes in high-CO2, acidified oceans, Mar Ecol 20: 373, 295–302.

Jordaan A, Kling LJ (2003) Determining the optimal temperature range for Atlantic cod (Gadus morhua) during early life. In: Browman HI, Skiftesvik AB, eds. The Big Fish Bang Proceedings of the 26th Annual Larval Fish Conference. Bergen, Norway: Inst Mar Resrch. pp45–62.

Jörger KM, Stöger I, Kano Y, Fukuda H, Knebelsberger T, Schrödl M (2010) On the origin of Acochlidia and other enigmatic euthyneuran gastropods, with implications for the systematics of Heterobranchia. BMC Evol Biol 10:323.

Katersky RS, Carter CG (2007) High growth efficiency occurs over a wide temperature range for juvenile barramundi *Lates calcarifer* fed a balanced diet. Aquaculture 272: 444–450.

Knowlton AL, Highsmith RC (2000) Convergence in the time-space continuum: a predator-prey interaction. Mar Ecol Prog Ser 197: 285-291.

Kress A (1975) Observations during embryonic development in the genus *Doto* (Gastropoda: Opisthobranchia). J Mar Biol Assoc United Kingdom 55:691-701.

Lalli CM, Conover RJ (1973) Reproduction and development of *Paedoclione doliiformis*, and a comparison with *Clione limacine* (Opisthobranchia: Gymnosomata). Mar Biol 19:13-22.

Llodra ER (2002) Fecundity and life-history strategies in marine invertebrates. Adv Mar Biol 43: 87–170.

McMahon JJ, Summers WC (1971) Temperature effects on the development rate of squid (*Loligo pealei*) embryos. Biol Bull 141: 561–567.

Melzner F, Gutowska MA, Langenbuch M, Dupont S, Lucassen M, Thorndyke MC, Bleich, M., Pörtner, HO (2009) Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosciences 6: 2313–2331.

Moran AL, Emlet RB (2001) Offspring size and performance in variable environments: field studies on a marine snail. Ecol 82:1597-1612.

Moran AL, Woods HA (2007) Oxygen in egg masses: interactive effects of temperature, age, and egg-mass morphology on oxygen supply to embryos. J Exp Biol 210:722–73.

Morse MP (1971) Biology and life history of the nudibranch mollusk *Coryphella stimpsoni* (Verrill 1879). Biol Bull 140:83-94.

Munday PL, Crawley N, Nilsson GE (2009) Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. Mar Ecol Prog Ser 388: 235–242.

Newell RC (1973) Factors affecting the respiration of intertidal invertebrates. Oxf Uni Press 13 (2): 513:528.

Palmer AR, Strathmann R (1981) Scale of dispersal in varying environments and its implications for life histories of marine invertebrates. Oecologia 48: 308-318.

Pelseneer P (1922) Sur un habitude de *Doris bilamellata*. Annales Soc Royl Zoo Belgie 53:28-32.

Perron FE, Turner RD (1977) Development, metamorphosis, and natural history of the nudibranch *Doridella obscura* Verrill (Corambidae: Opisthobranchia). J Exp Mar Biol Ecol 27: 171-185.

Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate change and distribution shifts in marine fishes. Science 308: 1912–1915.

Pianka ER (1970) On r and K selection. Amer Natur 104:592-597.

Pimentel MS, Trübenbach K, Faleiro F, Boavida-Portugal J, Repolho T & Rosa R (2012) Impact of ocean warming on the early ontogeny of cephalopods: a metabolic approach. Mar Biol. DOI: 10.1007/s00227-012-1991-9.

Plaut, I., Borut, A., Spira, M.E., 1995. Growth and metamorphosis of Aplysia oculifera larvae in laboratory culture. Marine Biology. 122:425-430.

Pörtner HO (2001) Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88:137–146.

Pörtner HO (2002) Environmental and functional limits to muscular exercise and body size in marine invertebrates athletes. Comp Biochem Physiol A 133:303–321.

Pörtner HO, Mark FC, Bock C (2004) Oxygen limited thermal tolerance in fish? Answers obtained by nuclear magnetic resonance techniques. Resp Physiol Neurobiol 141:243–260.

Pörtner HO, Langenbuch M, Michaelidis B (2005) Synergistic effects of increased CO_2, temperature and hypoxia on marine animals. J Geophys Res 110:C09S10.

Pörtner HO, Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315: 95–97.

Potts GW (1983) The respiration of *Onchidoris bilamellata* and *Archidoris pseudoargus* (Doridacea). J Mar Biol Ass UK 63: 399-407.

Przeslawski R (2004) Review of environmental effects on intertidal molluscan egg mass development and mortality. Molluscan Res. 24:43 -63.

Rafinesque CS (1814) *Précis des dé couvertes somiologiques ou zoologiques et botaniques*. Palermo 30.

Reiber CL, McMahon BR (1998) Progressive hypoxia's effects on the crustacean cardiovascular system: a comparison of the freshwater crayfish (*Procambarus clarkii*) and the lobster (*Homarus americanus*). J Comp Physiol. 168(3):168–176.

Rivkin RR (1991). Seasonal patterns of planktonic production in McMurdo Sound, Antarctica. Amer Zool 31:65-80.

Rosa R, Pimentel MS, Boavida-Portugal J, Teixeira T, Trübenbach K & Diniz MS (2012) Ocean warming enhances malformations, premature hatching, metabolic suppression and oxidative stress in the early life stages of a keystone invertebrate. PLOS One 7(6): e38282.

Rosa R, Seibel BA (2008) Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. Proc. Natl. Acad. Sci. 52:20776-20780.

Rosa R, Trueblood L, Seibel BA (2009) Ecophysiological influence on scaling of aerobic and anaerobic metabolism of pelagic gonatid squids. Physiol Biochem Zool 82:419-429.

Rosa R, Seibel BA (2010) Metabolic physiology of the Humboldt squid, Dosidicus gigas: Implications for vertical migration in a pronounced oxygen minimum zone. Prog. Oceanogr. 86:72-80.

Smith DA, Sebens KP (1983) The physiological ecology of growth and reproduction in *Onchidoris aspera* (Alder & Hancock) (Gastropode: Nudibranchia). J Exp Mar Bio Ecol 72: 287-304.

Solomon S, Quin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (2007) Climate Change 2007: The Physical Science Basis: Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge Univ. Press, Cambridge and New York.

Steer MA, Moltschaniwskyj NA, Jordan AR (2003) Embryonic development of southern calamari (*Sepioteuthis australis*) within the constraints of an aggregated egg mass. Mar Freshw Res 54: 217–226.

Stanwell-Smith D, Peck LS (1998) Temperature and embryonic development in relation to spawning and field occurrence of larvae of three Antarctic echinoderms.Biol. Bull. 194:44 -52.

Strathmann RR (1985) Feeding and non-feeding larval development and life-history evolution in marine invertebrates. Annu Rev Ecol Syst 16: 339-361.

Strathmann MF (1987) Opisthobranchia 268.302, *In* Strathmann MF (Ed). Reproduction and development of marine invertebrates of the northern Pacific coast. Univer Wash Press, Seattle, WA. 670.

Switzer-Dunlap M, Hadfield, MG (1979) Reproductive patterns of Hawaiian aplysiid gastropods. In: Reproductive ecology of marine invertebrates (S. E. Stancyk, ed.) 197-210. Univer South Carol Press, Columbia.

Switzer-Dunlap MH, Hadfield MG (1984) The effect of size, age, and recent egg laying on copulatory choice of the hermaphroditic mollusc Aplysia juliana. . Int J Invertb Reprod Develop 7:217–225.

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, et al. (2004) Extinction risk from climate change. Nature 427: 145–148.

Thompson, TE, 1958. The influence of temperature on spawning in *Adalaria proxima* (A & H) (Gastropoda Nudibranchia). Oihos 9: 246-252.

Thompson TE (1966) Studies on the reproduction of *Archidoris pseudoargus* (Rapp) (Gastropoda Opisthobranchia). Philosophical Transactions of the Royal Society of London (B) 250:344-373.

Thompson TE (1967) Direct development in a nudibranch, *Cadlina laevis*, with a discussion of developmental processes in the Opisthobranchia. J Mar Biol Assoc United Kingdom 47:1-22.

Thompson TE (1976) Nudibranchs. T.F.H. Publications, Inc., Ltd., Neptune City, NJ. 96.

Thorp JH, Covich AP (2001) Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego, CA, 315–399

Todd CD (1979) The population ecology of *Onchidoris hilamellata* (L.) (Gastropoda: Nudibranchia). J Exp Mar Biol Ecol 41, 213-255.

Todd CD (1981) The ecology of nudibranch mollusks. Oceanogr Mar Biol Annu 19:141–234.

Todd CD (1983) Reproductive and trophic ecology of nudibranch mollusks. In Russell-Hunter WD (Ed.). The Mollusca 6. Academic Press, NewYork, NY 225–259.

Todd CD (1987) Reproductive energetic and larval strategies of nudibranch mollusks: effects of ration level during the spawning pediod in *Onchidoris muricata* (Müller) and *Adalaria proxima* (A & H). Am Malac Bull 5: 293-301.

Todd CD, Doyle RW (1981) Reproductive strategies of marine benthic invertebrates: a settlement-timing hypothesis. Mar Ecol Prog Ser 4: 75-83.

Todd CD, Havenhand JN (1985) Preliminary observations on the embryonic and larval development of three dorid nudibranchs. Journal of Molluscan Studies 51:226-228.

Turner RL, Lawrence JM (1979) Volume and composition of echinoderm eggs: implications for the use of egg size in life history models. In, Reproductive ecology of marine invertebrates SE Stancyk, Univ South Carolina Press, Columbia. 25-40.

Vidal EAG, DiMarco FP, Wormuth JH, Lee PG (2002) Influence of temperature and food availability on survival, growth and yolk utilization in hatching squid. Bull Mar Sci 71: 915–931.

Vinagre C, Narciso L, Cabral HN, Costa MJ, Rosa R (2012) Coastal *versus* estuarine nursery grounds: Effect of differential temperature and heat waves on juvenile seabass, *Dicentrarchus labrax*. Est Coast Shelf Sci 109: 133-137.

Wägele H, Johnsen G (2001) Observations on the histology and photosynthetic performance of "solar-powered" opistobranchs (Mollusca, Gastropoda, Opistobranchia) containing symbiotic chloroplasts or zooxanthellae. Org. Divers. Evol. 1, 193–210.

Wagner D, Kahng SE, Toonen RJ (2009) Observations on the life history and feeding ecology of a specialized nudibranch predator (Phyllodesmium poindimiei), with implications for biocontrol of an invasive octocoral (Carijoa riisei) in Hawaii. J Exp Mar Bio Eco 372: 64–74.

Wang T, Overgaard J (2007) The heartbreak of adapting to global warming. Science 315:49–50.

Watt JL, Aiken RB (2003) Effect of temperature on development time in egg masses of the intertidal nudibranch, *Dendronotus frondosus* (Ascanius 1774) (Opisthobranchia, Dendronotacea). Northeastern naturalist 10(1):17-24.

Williams LG (1974) Veliger development in *Dendronotus frondosus* (Ascanius 1774) (Gastropoda: Nudibranchia). The Veliger 14:166-171.

Woods HA, Moran AL (2008) Temperature–oxygen interactions in Antarctic nudibranch egg masses. J Exp Bio 211: 798-804.

ANNEXES

Table 1 – Results of one-way ANOVA evaluating the effects of temperature on *A.maculata*'s adult survival.

	df	MS	F	*p*
Temperature (T)	3	82.11	6.15	0.004
Error	20	13.35		

Table 2 – Results of one-way ANOVA evaluating the effects of temperature on food intake of *A.maculata*'s adults.

	df	MS	F	*p*
Temperature (T)	3	1.39	18.90	0.000
Error	16	0.07		

Table 3 – Results of one-way ANOVA evaluating the effects of temperature on *A.maculata*'s spawning frequency (number egg masses/week).

	df	MS	F	*p*
Temperature (T)	3	9.29	21.82	0.000
Error	200	0.43		

Table 4 – Results of one-way ANOVA evaluating the effects of temperature on total number of egg masses.

	df	MS	F	*p*
Temperature (T)	3	157.89	9.47	0.01
Error	8	16.67		

Table 5 – Results of two-way ANOVA evaluating the effects of embryonic stage and temperature on capsule volume.

	df	MS	F	*p*
Stage (S)	1	0.11	214.4	0.000
Temperature (T)	3	0.01	16.47	0.000
S x T	3	0.01	26.09	0.000
Error	562	0.00		

Table 6 – Results of two-way ANOVA evaluating the effects of embryonic stage and temperature on embryo volume.

	df	MS	F	*p*
Stage (S)	1	0.00	57.37	0.000
Temperature (T)	3	0.00	15.74	0.000
S x T	3	0.00	12.74	0.000
Error	562	0.00		

Table 7 – Results of two-way ANOVA evaluating the effects of embryonic stage and temperature on number of embryos per capsule.

	df	MS	F	*p*
Stage (S)	1	1,055.25	717.55	0.000
Temperature (T)	3	122.02	82.97	0.000
S x T	3	92.50	62.89	0.000
Error	532	1.47		

Table 8 – Results of one-way ANOVA evaluating the effects of temperature on veliger's length.

	df	MS	F	*p*
Temperature (T)	3	0.02	77.58	0.000
Error	146	0.00		

Table 9 – Results of one-way ANOVA evaluating the effects of temperature on blastula embryonic stage.

	df	MS	F	*p*
Temperature (T)	3	0.43	4.08	0.02
Error	16	0.11		

Table 10 – Results of one-way ANOVA evaluating the effects of temperature on gastrula embryonic stage.

	df	MS	F	*p*
Temperature (T)	3	0.87	4.33	0.02
Error	16	0.20		

Table 11 – Results of one-way ANOVA evaluating the effects of temperature on trocophore embryonic stage.

	df	MS	F	*p*
Temperature (T)	3	0.93	2.49	0.10
Error	16	0.38		

Table12 – Results of one-way ANOVA evaluating the effects of temperature on active-veliger embryonic stage.

	df	MS	F	*p*
Temperature (T)	3	1.89	2.79	0.08
Error	13	0.68		

Table 13 – Results of one-way ANOVA evaluating the effects of temperature on newly-hatched veliger embryonic stage.

	df	MS	F	*p*
Temperature (T)	3	3.06	7.61	0.00
Error	12	0.40		

Table 14 – Results of one-way ANOVA evaluating the effects of temperature on oxygen consumption on *A.maculata*'s adults.

	df	MS	F	*p*
Temperature (T)	3	0.02	18.06	0.00
Error	44	0.00		

Table 15 – Results of one-way ANOVA evaluating the effects of temperature on oxygen consumption on *A.maculata*'s embryos.

	df	MS	F	*p*
Temperature (T)	3	0.00	14.49	0.00
Error	16	0.00		