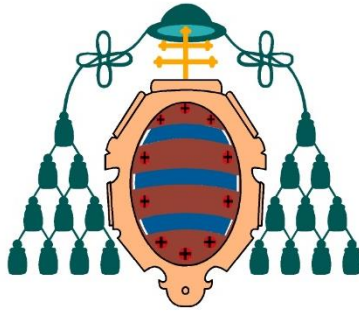


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UNIVERSIDAD DE OVIEDO

MASTER OF SCIENCE IN MARINE CONSERVATION

Máster Universitario en Conservación Marina

**ARTIFICIAL SELECTION ON LIFESPAN IN THE APPENDICULARIAN
OIKOPLEURA DIOICA.**

**Selección artificial sobre la duración de la vida en la apendicularia
Oikopleura dioica.**

MASTER THESIS

Trabajo de Fin de Máster

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Abstract

Artificial selection experiments are essential tools for understanding the variation patterns and the genetic basis of adaptation traits in evolutionary processes. Model organisms constitute an attractive target for several studies due to their simplified systems and short lifecycles. *Oikopleura dioica* (Fol, 1872) is a species of free-swimming pelagic tunicate widely distributed over temperate and warm waters. It is semelparous, dioecious and takes 5-8 days for completing their lifecycle at 15°C. *O. dioica* is important as a model species for developmental and phylogenetic studies, and the sampling and culturing methodology have been optimized. The aim of this Thesis is to test the possibility of conducting an artificial selection experiment to develop breeding lines with contrasting longevity as suggested in a previous study. To measure lifespan, two approaches were developed. One, based on the population size during reproduction (L_p) and another, based on direct timing of random individuals during reproduction (L_d). We successfully reared three consecutive generations, with an average lifespan of 6.9 ± 1.44 (mean \pm SD). The effect of artificial selection resulted non-significant ($p=0.873$ for L_p , $p=0.051$ for L_d) in the entire experiment, although a significant plastic response to the population density (d_0) prevailed over our selection treatments ($p=1.08 \times 10^{-5}$ for L_p , $p=0.003$ for L_d). The best model for L_p and L_d consisted on single straight lines fitted to the lifespan vs. d_0 data ($L_p = 0.0574 \times d_0 + 4.3019$; $L_d = 0.0586 \times d_0 + 4.436$). We hypothesize that lifespan extension dependent on population density may allow dispersal by ocean turbulence to escape from adverse environments.

Key words: Appendicularians, *Oikopleura dioica*, lifespan, artificial selection experiments, phenotypic plasticity, population density.

Introduction

Selection experiments are essential tools for understanding the variation patterns in evolution, and for assessing specific questions about the genetic basis of adaptation traits and evolutionary processes. Artificial selection is a type of selection experiment used in evolutionary research where the experimenter selects only the individuals that express a particular trait, allowing the trait propagation to the next generation (Fuller *et al.*, 2005). Artificial selection experiments are employed for different aims, for instance, the assessment of the symmetry of responses in a specific trait (Pitnick and Miller, 2000), which defines one of the main objectives of this work.

Artificial selection experiments are normally carried out on species of interest for natural sciences and biomedicine. These are model organisms, and constitute an attractive target for ecological, behavioural, developmental and genetic studies due to their simplified systems and short lifecycles (Nishino and Satoh, 2001). Some of the most common model species, *inter alia*, are *Drosophila melanogaster* for genetic studies (Reiter *et al.* 2001), *Caenorhabditis elegans* for genetics and biomedical researches (Kaletta and Hengartner, 2006), and the clonal cladoceran *Daphnia spp.* for ecological and genetic studies (Ebert *et al.*, 1993). New species are currently being tested to expand the boundaries of the model organism's usage in science, this is the case of the appendicularian *Oikopleura dioica* (Fol, 1872).

O. dioica is a species of free-swimming pelagic tunicate from the class Appendicularia. This species is widely distributed over temperate and warm waters in the Atlantic, Pacific and Indic Oceans, as well as in the Mediterranean and Red Seas (Fenaux *et al.*, 1998; van der Land, 2001). *O. dioica* is characterized by its morphology, which consists basically on a trunk and a chordate tail. The former secretes a filtering structure, the house, composed of a mucopolysaccharid complex and influences the buoyancy and feeding processes of these organisms (Körner, 1952; Fenaux, 1998). The latter is a muscular tail which appendicularians move periodically and sinusoidally to pump the water into the mucous house filters, generating a water flow from which *O. dioica* will obtain the food resources (Fenaux, 1986).

O. dioica is a semelparous species, with a single episode of reproduction before death; and has separate sexes (hence its name). It only takes from five to eight days for their lifecycle to be completed at 15 degrees Celsius (°C) (Lobón *et al.*, 2011; Martí-Solans *et al.* 2015), which is a lifespan shorter than the average of some invertebrates of similar size, that lives around 30 days in controlled conditions. This species is very important as a model species for developmental and

phylogenetic studies (Nishino and Satoh, 2001; Nishida, 2008), and over the years, the methodology of sampling and culturing this species have been optimized, bringing the possibility to maintain appendicularians for several generations (Bouquet *et al.* 2009, Martí-Solans *et al.* 2015).

In the first genetic study of life history traits in a pelagic tunicate, Lobón *et al.* (2011) explored the narrow sense heritability as well as the genetic correlation among morphological and life history traits in *O. dioica*. Using a dam-sire, half-sib breeding design, they measured a very high heritability for the lifespan ($h^2=0.89 \pm 0.47$, mean \pm SD), what pointed to the potential of using *O. dioica* for ageing research. The aim of this Thesis is to test the possibility of conducting an artificial selection experiment to develop breeding lines with contrasting longevity, as suggested by Lobón *et al.* (2011).

Materials and methods

Collection and preparation of the wild population. -The natural population was sampled at the *El Musel* Gijón's harbour by using a WP2 plankton net with a 40 cm mouth diameter, 50 μ m mesh size with an acrylic, closed cod end (Acuña *et al.*, 1994, Lobón *et al.* 2011). Only the healthiest individuals were transferred one-at-a-time to 4-liter, PYREX® Griffin beakers filled with seawater pre-screened through a 30 μ m Nitex sieve (30FSW) using wide bore serological polystyrene pipettes. The beakers were placed in a cold room set at $17.08\pm 0.45^\circ\text{C}$ (mean \pm SD) and 12:12 hours photoperiod and maintained following procedures in Bouquet *et al.* (2009) and Martí-Solans *et al.* (2015). Approximately 3 g of activated charcoal pellets (granular 4-8 mesh, C2764, Sigma-Aldrich) and 3 pearls of 1-Hexadecanol (258741-500G, Sigma) were added to each beaker to preserve water quality and reduce surface tension, respectively. Suspension and buoyancy of the specimens was achieved by means of methacrylate paddles (9 cm \times 27 cm) maintained in continuous rotation at 10 rpm with electric motors. Appendicularians were transferred to new beakers filled with fresh 30FSW and algal suspension every two days until maturation.

Animals were fed with an algal mixture made of *Isochrysis galbana* and *Nanochloropsis gaditana* with a concentration of 100 $\mu\text{gC L}^{-1}$ (as in Lobón *et al.* 2011). Our phytoplankton species were different from those of Bouquet *et al.* (2009), Lobón *et al.* (2011), and Martí-Solans *et al.* (2015), except for *I. galbana*. However, our algal mixture supported healthy populations of actively reproducing *O. dioica*. For calculation of the carbon concentration we used equations in Strathmann (1967) for non-diatom microalgae (3-5 μ m, *I. galbana*; 1-3 μ m, *N. gaditana* in

diameter). Our mixture consisted of 60% *I. galbana*, and 40% of *N. gaditana* by carbon, equivalent to ca $15-18 \times 10^6$ cells ml^{-1} of *I. galbana*, and $50-65 \times 10^6$ cells ml^{-1} of *N. gaditana*.

To start the artificial selection experiment, 60 sexually mature and healthy specimens from the natural population were isolated and separated by sexes into 250-ml beakers filled with seawater filtered through 0,2 μm MILLEX®GP filters, until the animals spawned their gametes and died. To achieve the simultaneous fertilization, the contents of both male and female beakers were mixed and stirred with 10 rpm paddles during 15-20 minutes approximately, what optimizes fertilization at 17 °C (Nishida, 2008). This step sets the start of the experiment. The 250-ml beakers containing the gametes were then diluted into a 4-liter beaker containing 30FSW and the algal mixture as in the preceding section. These animals represent the initial, wild population (F_0). The second day, 200 healthy juveniles inside houses were randomly transferred to each of seven beakers to establish the selection lines. Three of them were selected for short lifespan, another three for long lifespan, and the remaining population was used to measure lifespan in the wild population.

Measurement of the lifespan. -To measure lifespan we developed two different approaches. One of them based on following the population size during reproduction (L_p) and another based on direct timing of the reproduction of a randomly selected group of individuals (L_d). L_p was calculated as

$$L_p = \frac{\sum_{i=0}^{\infty} (n_i - n_{i+1}) \times [(t_i + t_{i+1}) / 2]}{n_0} \quad (1)$$

where n_0 is the population size at the start of reproduction, n_i is the population size at time t_i , $n_i - n_{i+1}$ is the number of individuals which reproduced during the time interval from t_i to t_{i+1} , and $(t_i + t_{i+1}) / 2$ is the average time at which those individuals reproduced (Fig. 1A). To calculate L_d for one population, we randomly selected up to 30 males and up to 30 females right before reproduction, isolated them, determined the exact moment of spawning of each individual, and averaged those measurements to calculate a mean lifespan.

Artificial selection. -Artificial selection of parental individuals was done according to the timing of their reproduction. For the short lifespan lines, we selected up to 20 males and up to 20 females of the earliest maturing appendicularians, depending on the total number of available individuals. A similar procedure was applied for the long lifespan lines, but selecting the latest maturing individuals. We measured the lifespan of each parental and calculated a mean lifespan for the parentals as for L_d above (“Selection” in Fig. 1B and C).

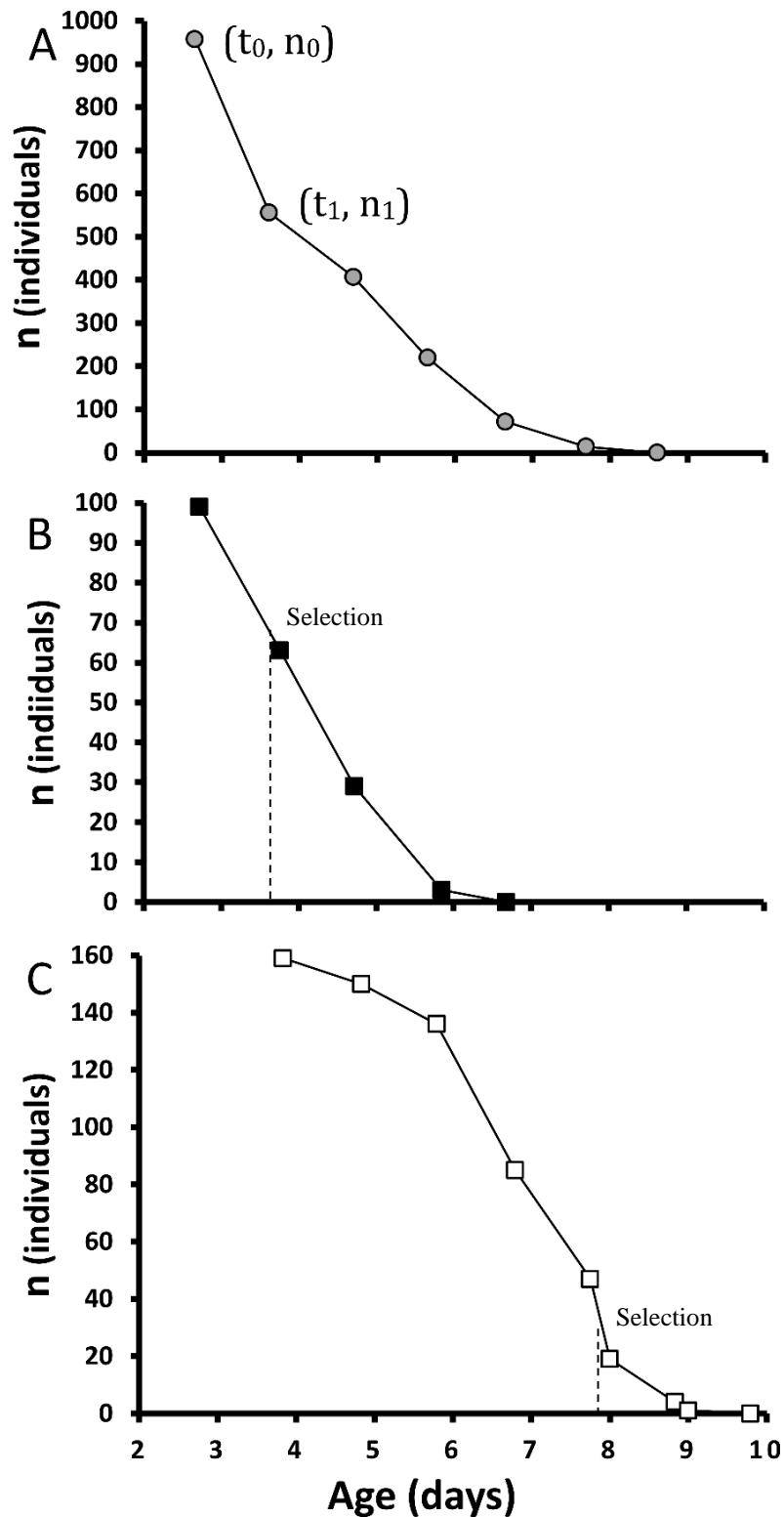


Figure 1. Time course of the number of appendicularians from beginning to end of their reproduction in three representative cohorts. **A**, in the wild population; **B**, in the F_2 of one line of the short lifespan treatment; **C**, in the F_2 of one line of the long lifespan treatment. Letters in panel A indicate terms for calculation of L_p in equation 1. Dashed lines in panels B and C indicate the moment of selection of parentals for the next generation.

Data analysis. - Narrow-sense heritability (h^2) was calculated for each population and generation using Falconer's Proband Method (cited in Roff, 2012) for comparison with that of Lobón *et al.* (2011). h^2 was calculated as

$$h^2 = \frac{R}{S} = \frac{\mu_R - \mu_P}{\mu_S - \mu_P} \quad (2)$$

where μ_P , μ_R and μ_S are the mean lifespans of population, the offspring and the selected parentals, respectively, $R = \mu_R - \mu_P$ is the response to selection and $S = \mu_S - \mu_P$ is the selection differential.

Lifespan comparisons among long and short lifespan lines were done using a repeated-measures ANOVA with treatment (short/long lifespan) as fixed factor, and population (replicated lines) as blocking factor. The initial reproduction density (d_0), calculated as the population size at the start of reproduction (n_0) divided by the total water volume in each beaker (4 L), was also included as covariate in a repeated-measures ANCOVA. Analyses were performed with the free software for statistical computing and graphics R (R Core Team 2013).

Quinn and Keough, (2002) point to a problem of carryover effects in these kind of designs, where the repeated measures are not independent, i.e. the value of one measure depends on the value of the previous one. This implies the assumption of the absence of accumulative effects across generations in our experimental design, an assumption which is at odds with the outcome of a typical selection experiment. However, Quinn and Keough (2002) also stated that the absence of carryover effects does not necessarily correspond to an absence of correlation between consecutive treatments in a repeated-measures sequence (in our case, between consecutive generations in the replicated populations), and that there will always be a correlation between repeated measures, being the correlation's nature the main determinant of the analysis strategy for these type of designs.

Results

We successfully reared three consecutive generations of *Oikopleura dioica* under artificial selection, with an average lifespan of 6.9 ± 1.44 days (mean \pm SD; N=1187). For the artificial selection experiment, we selected the earliest and the latest maturing individuals for the short and long lifespan parental lines, as indicated in Figure 1B and C, respectively. The selection differential, defined as the difference between the mean lifespan of the selected parentals and the mean lifespan of the population, resulted in concordance with the direction of selection (Fig. 2).

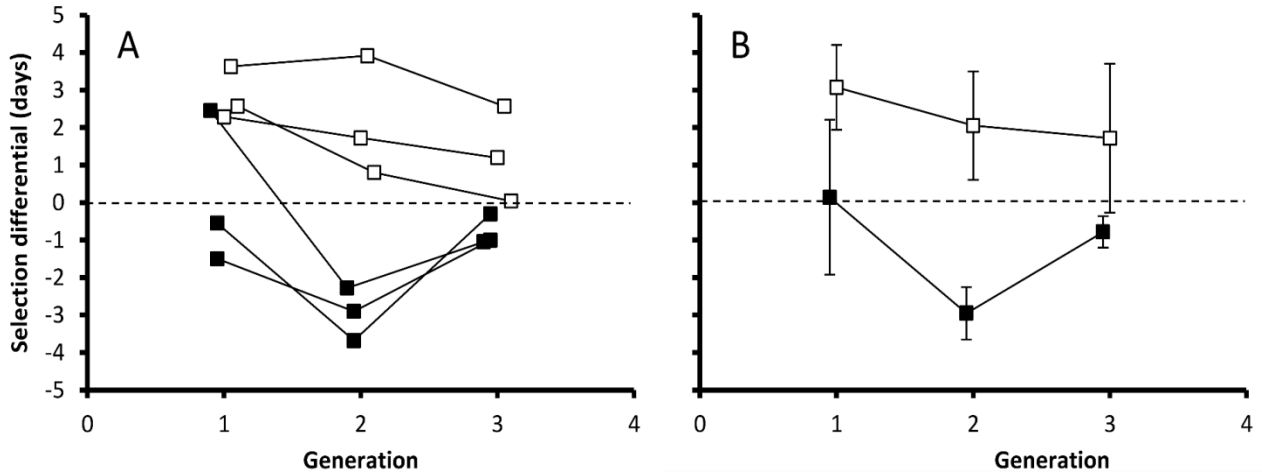


Figure 2. Selection differentials of the artificial selection experiment. Symbols correspond to lines selected for short lifespan (dark) and long lifespan (light). Lines join symbols corresponding to the same replicate population. **A**, calculated selection differentials; **B**, averages (\pm SD). The dashed line indicates the baseline from where selection differential is quantified.

However, artificial selection produced unexpected results. Both L_p and L_d varied in an apparently random pattern (Fig. 3) and the lifespan of the wild population ($L_p=4.47$ days, $L_d=5.38\pm 0.65$ days Fig. 3) was lower than most of the values in the selection lines. It is important to note that the third and last generation of the long lifespan lines coincided with a water contamination event, thus the observed lifespan might be altered. L_p and L_d were marginally non normal (Shapiro-Wilk test, $W=0.8725$, $p=0.0196$ for L_p and $W=0.9189$, $p=0.1238$ for L_d) although both were homoscedastic (Levene test: $F=0.1983$, $p=0.9571$ for L_p and $F=0.7384$, $p=0.6091$ for L_d). A repeated measures ANOVA test comparing selection treatments yielded differences which were non-significant for L_p ($p=0.873$, Table 1) and significant for L_d ($p=0.0335$, Table 1). However, the mean L_d for the short lifespan treatment (7.623 ± 1.675 days) was longer than that for the long lifespan treatment (6.339 ± 0.785 days). In other words, the responses were frequently opposed to the direction of selection (Fig. 3).

Table 1. ANOVA and ANCOVA results for lifespan estimations based on the population size L_p , and for lifespan estimations based on the direct timing of the reproduction of the population L_d . Significance level = 0.05.

		Source	df	SS	MS	Error term	F	P
ANOVA	L_p	Treatment	1	0.007	0.007	Populations	0.029	0.873
		Populations	4	0.9577	0.2394			
		Within	12	26.67	2.223			
	L_d	Treatment	1	7.413	7.413	Populations	10.12	0.0335
		Populations	4	2.93	0.732			
		Within	12	24.44	2.036			

		Source	df	SS	MS	Error term	F	P
ANCOVA	L_p	Treatment	1	0.007	0.007	Populations	0.035	0.863
		Density	1	22.391	22.391	Within	57.56	1.08×10^{-5}
		Populations	3	0.5941	0.198			
		Within	11	4.279	0.389			
	L_d	Treatment	1	7.413	7.413	Populations	9.969	0.051
		density	1	13.73	13.734	Within	14.12	0.003
		Populations	3	2.231	0.744			
		Within	11	10.7	0.973			

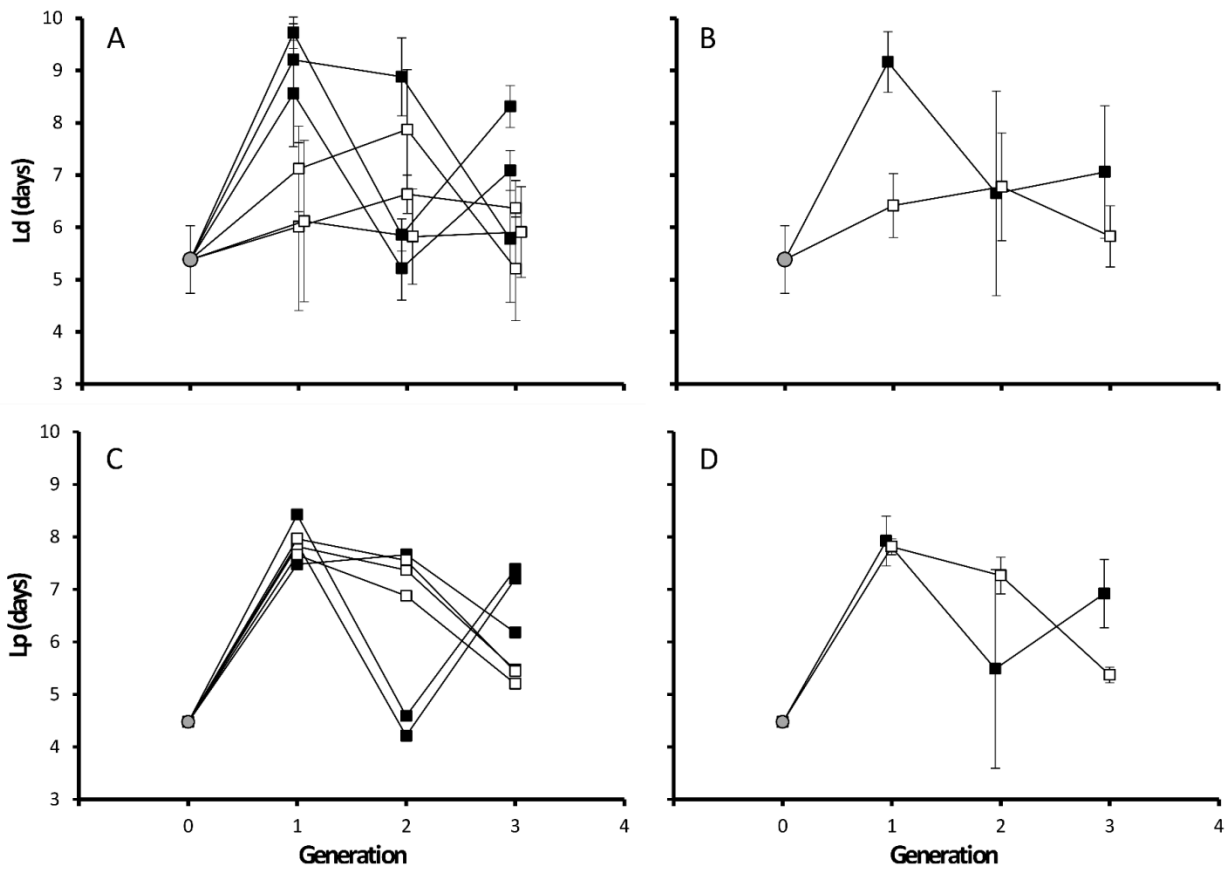


Figure 3. Result of the artificial selection experiment in *O. dioica*. Symbols correspond to lines selected for short (filled) and long (empty) lifespan and wild population (grey). Lines join symbols corresponding to the same replicate population. **A**, mean (\pm SD) observed lifespan (L_d); **B**, mean (\pm SD) treatmentwise L_d ; **C**, population lifespan (L_p); **D**, average (\pm SD) treatmentwise L_p .

Furthermore, heritabilities (h^2) in our experiment, calculated as the response to selection relative to the selection differential, exhibited atypical values for most of the populations from generation to generation (Fig. 4, Appendix Table 1 and 2). To summarize, the strong response to

selection expected from the high heritability measured by Lobón *et al.* (2011) for this trait never occurred in our experiment.

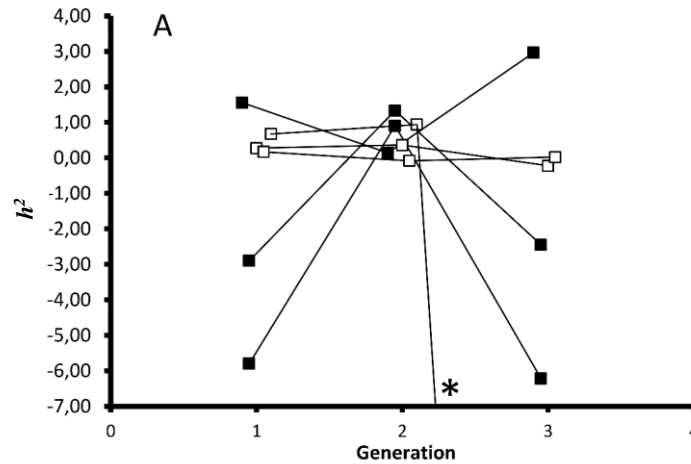


Figure 4. Narrow sense calculated heritabilities h^2 in the artificial selection experiment. Symbols correspond to lines selected for short lifespan (dark) and long lifespan (light). Lines join symbols corresponding to the same replicate population. Asterisk stands for an outlying value (see Appendix Table 1).

There was some consistency among our alternative lifespan measurements (L_p and L_d), which were positively correlated (Pearson correlation coefficient = 0.69), with a highly significant least-squares regression ($L_p = (0.677 \pm 0.158) \times L_d + (2.007 \pm 1.109)$; $R^2 = 0.492$; $F_{1,17} = 18.44$, $p = 0.0005$) although the swarm of points suggested a curved relationship (Fig. 5).

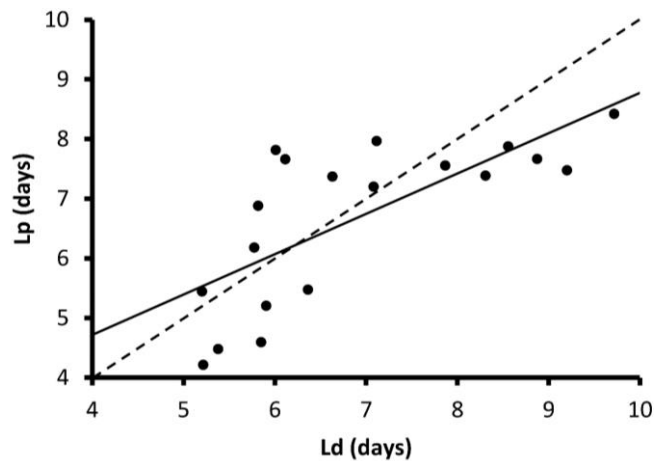


Figure 5. Relationship between the alternative lifespan measurements L_p and L_d . The solid line represents the least-squares fit to a straight line ($L_p = (0.677 \pm 0.158) \times L_d + (2.007 \pm 1.109)$; $R^2 = 0.492$; $F_{1,17} = 18.44$, $p = 0.0005$). The dashed line represents the perfect, 1:1 correspondence among both variables.

Recently, Subramaniam *et al.* (2014) reported on the existence of phenotypic plasticity in some life story traits of *O. dioica*. They identified growth arrest in response to high population

densities and dietary limitations, what consequently extended the lifespan of *O. dioica* at least three-fold. In our experiment, we counted the number of individuals several times during the course of reproduction, including an initial count (n_0 , Fig. 1), which we can use as a proxy for population density (d_0). We included d_0 as covariate in a repeated measures ANCOVA and tested again for differences among treatments, to account for potential plasticity as a response for population density. There were non-significant differences in the slopes of the lifespan vs. d_0 lines neither for L_p nor L_d (F-test on the interaction: selection treatment* d_0 , $F_{1-10} = 0.027$ and $p = 0.884$ for L_p ; and $F_{1-10} = 6.675$ and $p = 0.123$ for L_d).

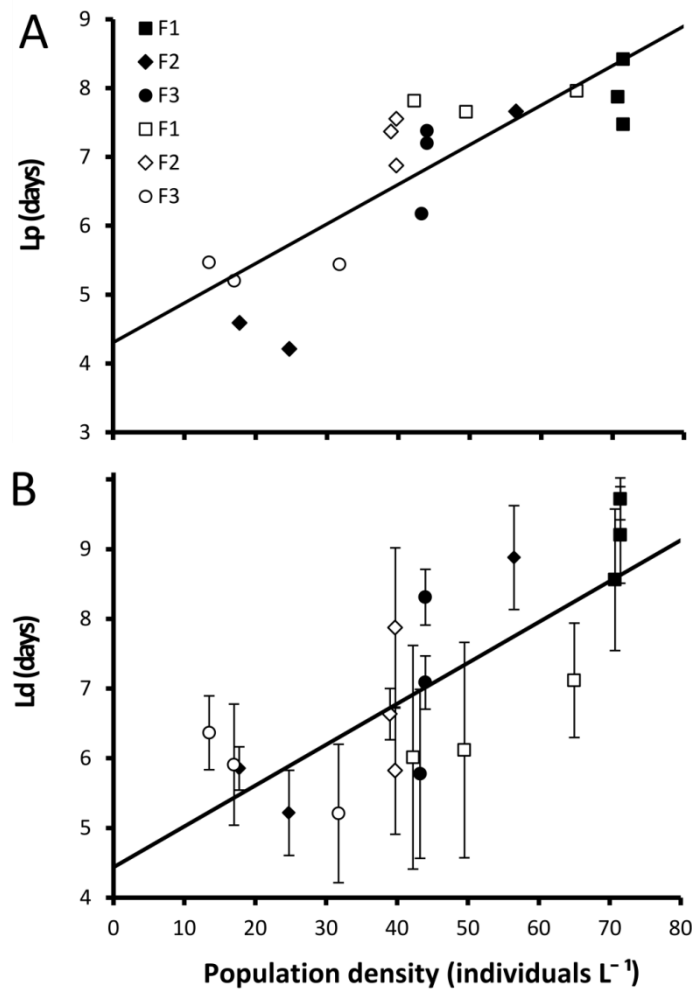


Figure 6. Effect on the lifespan of the population density at start of reproduction. **A**, L_p vs d_0 . The line is the least squares fit, $L_p = (0.0574 \pm 0.0096) \times d_0 + (4.3019 \pm 0.4514)$, $R^2 = 0.671$, $F_{1-16} = 35.74$, $p = 1.929 \times 10^{-5}$. Filled and empty symbols correspond to the short and long lifespan lines, respectively. **B**, L_d vs d_0 . Same convention as for panel A. The least squares regression yields $L_d = (0.0586 \pm 0.0127) \times d_0 + (4.436 \pm 0.596)$, $R^2 = 0.545$, $F_{1-16} = 21.34$, $p = 0.0003$. The population density (d_0) has been calculated as the population size at the start of reproduction, n_0 , divided by the total water volume in each beaker, 4 L.

According to ANCOVA there were not significant differences in elevation among selection treatments, confirming the absence of effect of artificial selection in L_p and L_d ($p=0.863$ and $p=0.051$, Table 1, Fig. 6A and B, respectively). However, d_0 had a large and significant effect on lifespan ($p=1.08\times 10^{-5}$ and $p=0.003$ for L_p and L_d , respectively, Table 1), thus the best model for L_p and L_d was one where a single straight line is fitted to the lifespan vs. d_0 data ($L_p = (0.0574\pm 0.0096)\times d_0 + (4.3019\pm 0.4514)$, $R^2 = 0.671$, $F_{1,16} = 35.74$, $p = 1.929\times 10^{-5}$, Fig. 6A; $L_d = (0.0586\pm 0.0127)\times d_0 + (4.436\pm 0.596)$, $R^2 = 0.545$, $F_{1,16} = 21.34$, $p = 0.0003$, Fig. 6B). This suggests that there is no effect of artificial selection on lifespan.

Discussion

Artificial selection for short and long lifespan in the appendicularian *Oikopleura dioica* did not confirm the prediction by Lobón et al. (2011) of potentially strong selection potential in this trait. Instead, there was a plastic response to the population density which prevailed over our selection treatments (Fig. 6, Table 1), rendering outlying heritabilities (Fig. 4, Appendix Table 1 and 2). The population density is known to increase appendicularians lifespan by stimulating growth arrest at a high population densities or when food is limited (Subramaniam *et al.*, 2014). However, the wild population lifespan was lower than most of the values in the selection lines, which can hold an epigenetic transgenerational response to a previous environmental situation, in other words epigenetic memory (Mirbahai and Chipman, 2014), which manifested itself simultaneously with our starting generation F_0 .

Phenotypic plasticity is defined as the ability of a single genotype to produce different phenotypes in response to the environmental conditions (the so-called *reaction norms*, West-Eberhard, 1989), and represents the solution to the problem of coping with the natural environmental variability at time scales comparable with the ontogenetic process. Heritability, however, is referred to the proportion of variation in a trait when it is under genetic influence (Stearns, 1992). These two concepts, taken in a restricted sense, cannot be discriminated when studied under the same environmental conditions and need more complex and longer-term experiments to establish their relation and the heritability of reaction norms (Stearns, 1992).

If phenotypic plasticity prevails in this trait, why did Lobón et al. (2011) measure such a high heritability for the lifespan? They carried out their experiment in only one generation, by growing each half-sib batch in a beaker for one day, and then transferring 10 randomly selected individuals to a new beaker to continue the experiment. They showed some variability in the number of eggs that a female *O. dioica* is able to produce (122.5 ± 87.2 mean \pm SD, Lobón et al,

2011). Moreover, growth arrest in this species only happens after early exposure to high population densities (not more than 4 days, Subramaniam *et al.*, 2014). Therefore, we can presume that the density of half-sibs in the same beaker during the first day of experiment in Lobón *et al.* (2011) may have influenced their lifespan, inflating the variability among half-sib families and leading to severe overestimation of the heritability for this trait.

What is the advantage of such a plastic response of the lifespan to population density? The largest appendicularian densities in our experiment (71.5 individuals L⁻¹, Appendix Table 1) are comparable to the highest recorded densities in the open ocean (≤ 26 individuals L⁻¹ found by Seki, 1973; and >100 individuals L⁻¹ found by Dagg *et al.*, 1996), thus our experimental range seems consistent with conditions in nature. Population bursts (or "red tides" sensu Seki, 1973) seem to be a recurrent feature of the life cycle of these animals (Seki, 1973; Nakamura, 1998; Fernández and Acuña, 2003; Lobón *et al.* 2013), usually as a response to high phytoplankton productivities and standing stocks (Acuña, 1992; Dagg *et al.*, 1996). During these episodic population bursts we may expect intense intraspecific competition for limited resources, clearly not the best environment to leave offspring. Bearing this in mind, we hypothesize that lifespan extension during high density episodes may allow dispersal by ocean turbulence, allowing escape from a high-density hypercompetitive environment. Assuming standard horizontal turbulence values in the ocean surface, the population density of an appendicularian patch would decrease proportionally to the square of the dispersal distance. According to our results, the minimum lifespan, given by the 0-intercept of the regression line in Figure 6A, is 4.3 days, while the maximum is 8.4 days. What does a plastic difference of 4.1 days mean in terms of dispersal scales? We can respond to this question with a rough estimation based on our knowledge of ocean turbulence.

The 2D dispersion time in a turbulent fluid (t , measured in seconds) can be expressed as

$$t = \frac{L^2}{D_t} \quad (3)$$

where L^2 is the square of the distance (assuming a circular patch, the distance would be the radius of that circle) and D_t is the oceanic turbulent dispersion coefficient ($\text{m}^2 \text{s}^{-1}$) (Thorpe, 2007). If we solve for L , we arrive at

$$L = (t \times D_t)^{1/2} \quad (4)$$

There is a difference of 4.1 days (354 594.24 seconds) in lifespan among individuals reared at low and high densities, and D_t may vary in the ocean between 1 and 10 m² s⁻¹ (Okubo, 1971). By applying these figures to equation 4, the dispersal scale during a lifetime would vary between 595.48 meters at low population densities and 1883.07 meters at high population densities.

In summary, the results of our study not only disagree with the potentially strong selection potential for the lifespan in *O. dioica* predicted by Lobón *et al.* (2011) due to its high phenotypic plasticity dependent on the population density; but also pointed out the influence of population density in the lifespan extension as a dispersal mechanism to escape from a high-density hypercompetitive environment. Indeed, these results can open the doors to a fascinating field of study to answer several emerging questions from our hypothesis: Which advantages can come from phenotypic plasticity? How are reaction norms inherited? How these animals perceive the population density in their surrounding environment? More research to answer these questions would be desirable in the future.

Conclusions

Artificial selection experiments for short and long lifespan in the appendicularian *Oikopleura dioica* resulted unviable due to the prevalence of phenotypic plasticity on this trait.

Population density, which is known to extend the lifespan in *O. dioica* by stimulating growth arrest at high population densities or dietary limitations, had a significant effect on our experiment. Any future research in this direction should take into account the population density and ensure its control.

We hypothesize that lifespan extension dependent on population density may allow dispersal by ocean turbulence to escape from high-density hypercompetitive environments. If feasible, this hypothesis could open the doors to a fascinating field of study to answer several emerging questions related to the advantages of phenotypic plasticity, the inheritance of reaction norms, or even the perception approaches of these animals to perceive the population density in the surrounding environment.

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Appendix

Table 1. Summary table for the artificial selection experiment variables. n_0 , indicates the initial number of individuals at the beginning of the reproduction; d_0 indicates the initial appendicularian density in the beakers at the beginning of the reproduction, calculated as n_0 divided by the total water volume in each beaker, 4 L. Population lifespans L_p and L_d are presented in days.

Treatment	Replicate	Generation	n_0	d_0	L_p	L_d (SD)	Selection differential	h^2
Wild	0	0	958	239.5	4.474	5.382 (0.650)	-	-
Short	1	1	283	70.75	7.874	8.560 (1.015)	-0.548	-5.796
	2	1	286	71.5	7.475	9.203 (0.692)	2.458	1.555
	3	1	286	71.5	8.421	9.720 (0.300)	-1.496	-2.898
	1	2	99	24.75	4.211	5.217 (0.610)	-3.680	0.908
	2	2	226	56.5	7.661	8.878 (0.745)	-2.279	0.143
	3	2	71	17.75	4.589	5.853 (0.310)	-2.902	1.333
	1	3	176	44	7.198	7.086 (0.382)	-0.301	-6.211
	2	3	173	43.25	6.178	5.777 (1.213)	-1.047	2.962
	3	3	176	44	7.383	8.310 (0.402)	-1.004	-2.447
Long	1	1	169	42.25	7.815	6.013 (1.604)	2.287	0.276
	2	1	198	49.5	7.658	6.117 (0.821)	4.365	0.168
	3	1	260	65	7.962	7.118 (1.545)	2.571	0.675
	1	2	156	39	7.367	6.633 (0.366)	1.723	0.360
	2	2	159	39.75	6.877	5.821 (1.148)	3.629	-0.082
	3	2	159	39.75	7.553	7.870 (0.912)	0.799	0.942
	1	3	54	13.5	5.471	6.366 (0.530)	1.195	-0.224
	2	3	68	17	5.202	5.908 (0.993)	3.914	0.022
	3	3	127	31.75	5.440	5.207 (0.868)	0.039	-67.974

Appendix

Table 2. Population averages treatmentwise for the artificial selection experiment variables. Population lifespans Lp and Ld are presented in days.

Treatment	Parameter	Generation	Mean (SD)
Wild	Lp (days)	0	4.474
	Ld (days)	0	5.382 (0.650)
Short	Lp (days)	1	7.923 (0.475)
		2	5.487 (1.892)
		3	6.920 (0.649)
	Ld (days)	1	9.161 (0.581)
		2	6.649 (1.956)
		3	7.058 (1.267)
	h^2	1	-2.380 (3.702)
		2	0.795 (0.603)
		3	-1.899 (4.611)
	Selection differential	1	0.138 (2.064)
		2	-2.954 (0.702)
		3	-0.784 (0.419)
Long	Lp (days)	1	7.812 (0.152)
		2	7.266 (0.349)
		3	5.371 (0.147)
	Ld (days)	1	6.416 (0.610)
		2	6.775 (1.032)
		3	5.827 (0.584)
	h^2	1	0.373 (0.267)
		2	0.407 (0.513)
		3	-22.725 (39.187)
	Selection differential	1	3.074 (1.126)
		2	2.050 (1.443)
		3	1.716 (1.989)