Range expansions across ecoregions: interactions of climate change, physiology and genetic diversity

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(A) ABSTRACT

Aim Climate change is expected to drive range shifts among a wide array of organisms. Non-indigenous species (NIS) provide a unique opportunity to witness the establishment of range boundaries in a way that cannot be directly observed for native species. Recent studies have indicated that climate change facilitates biological invasions at local scales. However, the generality of these effects is unclear, as there is a dearth of comparative studies that assess how rapid environmental change affects species ranges across taxa and biogeographic provinces.

Location South African and other coastlines of the world.

Methods We first studied the distribution of shallow-marine benthic organisms along the South African coastline, which consists of several dissimilar biogeographic provinces. We then obtained DNA sequence data from a suite of co-occurring NIS from along the studied coastline and compared these data with available genetic information from other regions of the world. Subsequently, we conducted physiological experiments and assess how thermal tolerance related to species ranges distribution. Finally, we analysed long-term seawater temperature records and compared these with past changes in range size and abundance patterns.

Results NIS with different thermal tolerances and range distributions have expanded their ranges and increased in abundance along the studied coastlines. Most haplotypes of the studied NIS in South Africa were shared with other regions, indicating that the studied populations were representative of other regions within the introduced range. Long-term records showed that seawater temperature regimes have recently changed along the studied coast.

Main conclusions This study provides empirical evidence that NIS, regardless of their thermal tolerance, range size and genetic variability, are expanding their ranges and increasing in abundance. This range expansion trend is concurrent with changes in seawater temperature, which suggests that climate change fosters NIS spread and abundance across multiple spatial scales, contributing towards global biotic homogenization.

(A) KEY WORDS: Ascidians, biogeography, invasive species, ecotones, naturalization, non-native, performance curve, population expansion, thermal sensitivity.

(A) INTRODUCTION

Species ranges are historically affected by climatic fluctuations (Roy *et al.*, 2001) that may result in latitudinal range expansions and contractions, with major changes in species borders and ecotones (Parmesan *et al.*, 2005). Temperature is one of the most important abiotic factor determining the distribution of the world's biota (Belanger *et al.*, 2012), as it influences physiological processes and species interactions across a wide range of taxa (Somero, 2012). For example, temperature affects the ability of propagules and juveniles to disperse and complete development and thus, may determine geographic ranges of species (Bonte *et al.*, 2008) and intra-specific genetic lineages (Teske *et al.*, 2008). Consequently, temperature and latitudinal shifts in range boundaries are inextricably linked.

Our perception of species ranges becomes considerably more complex when non-indigenous species (NIS) are involved, as they do not share an evolutionary history with the native community and, once introduced, establish new range limits that can remain labile for decades (e.g. Crisp & Southward, 1959). Thus, NIS provide an unparalleled opportunity to observe the establishment of species ranges in a way that cannot be directly observed for native species with long established boundaries (Sax *et al.*, 2007). Understanding how NIS ranges are determined, therefore, represents a rich source of knowledge, especially at a time when human-induced climate change and disturbances are expected to alter species' ranges worldwide (Walther *et al.*, 2009). However, it is important to be aware that NIS ranges may be (at least initially) set in ways that are fundamentally different from the natural boundaries of native species.

92 The introduction of NIS is generally attributed to a transient window of
93 opportunity (Davis *et al.*, 2005). Each new colonization event results from the arrival of
94 only a tiny fraction of the source population (founder event) and that fraction will carry
95 only a subset of overall genetic diversity (Sakai *et al.*, 2001). However, single
96 colonisations are rare and multiple introductions may be more common (e.g. Kolbe *et al.*,
97 2004), allowing introduced populations to escape bottleneck effects. Once a NIS is
98 naturalized, the next step is the invasive period. This generally includes a sudden
99 geographic expansion or a series of salutatory expansions, after which the rate of spread
100 drops and range size eventually stabilizes within new boundaries (Prentis *et al.*, 2008).
101 Climate change is expected to alter temperature regimes and generate poleward
102 and upward range shifts of native species globally (Parmesan *et al.*, 2005). The
103 population dynamics and impacts of NIS will also respond to climate change (Walther *et*
104 *al.*, 2009), and recent research suggests that such change will disproportionately facilitate
105 NIS at local scales (Stachowicz *et al.*, 2002; Sorte *et al.*, 2010). However, the generality
106 of these effects is unclear as there is a dearth of comparative studies that assess how rapid
107 changes in environmental conditions affect species ranges across taxa and biogeographic
108 provinces.
109 We investigated historical range shifts of multiple co-occurring NIS across
110 divergent biogeographic coastal regions to understand the role of thermal limits, range
111 size, genetic signatures and climatic variability, in shaping and maintaining species
112 ranges. We began by documenting the distribution of shallow-marine benthic organisms
113 along a coastline comprising several biogeographic provinces. We then compared
114 regional and global genetic signatures of a suite of NIS. Subsequently, we investigated

115 the effects of temperature on individual performance of a subset of species to understand
116 the role of environmental filtering. Finally, we analysed long-term temperature records
117 for the studied coast and evaluated historical changes in species ranges and abundance.
118 Specifically, this research addressed the following questions:

119 1. Are the studied NIS similar in terms of range size and physiological limits?

120 2. Is the genetic composition of the studied populations representative of the
121 genetic pool of the global species range?

122 3. Is there evidence that NIS are expanding their ranges and increasing in
123 abundance? If so, could climate change be responsible for facilitating NIS success at
124 regional and global scales?

125 We inferred that given that the studied species are most likely adapted to different
126 temperature regimes, their temperature tolerance would differ. We predicted that
127 comparisons between regional and global genetic signatures would show similar
128 composition among different regions within the introduced range as a result of human-
129 mediated transport. We hypothesized that range expansions of multiple NIS would occur
130 across eco-regions, and that historical temperature variation is consistent with increases
131 in abundance and rate of spread of these NIS.

132

133 **(A) MATERIAL AND METHODS**

134 **(B) *Studied taxa***

135 Interest in marine NIS has increased not only because they have a great ability to
136 displace native species and alter ecosystem processes, but also because they have
137 economic impacts on human activities. Shipping and aquaculture activities are the main

138 vectors of marine species introductions worldwide (McQuaid & Arenas, 2009), which are
139 concentrated in harbours and marinas. As the dispersal capabilities, substratum
140 occupation strategies, and response to environmental factors vary widely among taxa, we
141 chose as our model system the Class Ascidiacea (Tunicata, Chordata), a group containing
142 conspicuous members of coastal benthic and fouling communities worldwide, including
143 key bioengineering species with disjunct distributions (e.g. Teske *et al.*, 2011). Ascidians
144 are sessile as adults and the motile stages (embryonic and lecithotrophic larval stages)
145 can last from just minutes to a few days, which allows for short-distance dispersal
146 (Millar, 1971). Therefore, transoceanic dispersal of these species is solely human
147 mediated.

148 **(B) *Study region***

149 The 3000 Km of the South African coastline contains multiple biogeographic
150 regions and a broad gradient in thermal conditions, from tropical waters on the east coast
151 to cool-temperate waters on the west coast (Emanuel *et al.*, 1992), which provide an ideal
152 system for examining mechanisms shaping species biogeography. The region is a
153 crossroad for several major transoceanic trading routes (Kaluza *et al.*, 2010) since the 10th
154 century (Yap & Man, 1996), and has an active aquaculture industry (Rius *et al.*, 2011).

155 **(B) *Surveys***

156 We surveyed all main harbours along the South African coastline, plus five
157 recreational marinas and an oyster farm (Fig. 1, see Table 1 for details). We chose these
158 sites because they cover the entire coastline and they include virtually all the main entry
159 points for NIS. We considered the three traditional major biogeographic provinces
160 proposed for South Africa, namely the west, south and east coasts (Fig. 1) (Stephenson &

161 Stephenson, 1972) (see details in Appendix S1). The surveys were conducted twice (2007
162 and 2009) during the austral winter (see details of sampling methodology and species
163 identification in Appendix S2).

164 **(B) *Genetic study***

165 Specimens of the widespread NIS *Clavelina lepadiformis*, *Ciona intestinalis*,
166 *Styela plicata*, *Microcosmus squamiger* (see details about these species in Appendix S3)
167 were collected from the same sites during the 2009 survey and in addition we obtained
168 samples of *S. plicata* and *M. squamiger* from Richard's Bay harbour (28°47'39"S,
169 32°04'45"E) (Fig. 1, Table S1). Sites where fewer than five individuals were found were
170 excluded from the analyses. Samples were collected by hand from harbour ropes or
171 floating pontoons and fixed in absolute ethanol. In addition, we obtained samples from
172 other biogeographic regions including individuals from Azores and Madeira (see details
173 in Table S2). To maximize information for other regions from Genbank, we targeted a
174 section of the mtDNA (cytochome oxydase subunit 1, i.e. COI). The smaller effective
175 population size and high mutation rate of mitochondrial markers make them extremely
176 useful for geographic genetic studies (Avise, 2009), particularly studies of biological
177 invasions (e.g. Pineda *et al.*, 2011). It has been shown that the mutation rate of mtDNA is
178 conservative enough to retain information on the origins and range expansion of
179 introduced populations (Rius *et al.*, 2008). We excluded GenBank COI sequences that
180 did not align with our haplotypes because they covered a different section of the target
181 gene or the final alignment was unacceptably short. Sequences were obtained using
182 primers described in Table S3 (see general genetic methods in Appendix S4) and aligned
183 in BioEdit v. 7.0.5.2 (Hall, 1999). We then used DnaSP v. 5.10 (Librado & Rozas, 2009)

184 to translate nucleotide sequences into amino acid sequences (using the ascidian mtDNA
185 code) and to determine the number of haplotypes and standard diversity indices
186 (haplotype and nucleotide diversities), and the number of unique haplotypes. Parsimony
187 haplotype networks were generated using the programme TCS v. 1.21 (Clement *et al.*,
188 2000), which creates an absolute distance matrix by calculating all possible pairwise
189 comparisons among haplotypes, considering a parsimony probability of 0.95.

190 **(B)** ***Effects of temperature on ontogenetic stages***

191 Temperature may not determine species ranges through its effects on adult
192 performance (Gilman, 2006), as other life stages may be more sensitive (Pineda *et al.*,
193 2012). Therefore, distributional ranges can be set by the tolerance levels of sexual and
194 asexual propagules rather than adult fitness. To test ontogenetic effects of temperature,
195 we studied the effects of seawater temperature on development of all pre-adult life-
196 history stages. We selected four different NIS (*Ciona intestinalis*, *Ascidiella aspersa*,
197 *Styela plicata* and *Microcosmus squamiger*) that have widespread distributions along the
198 world's coastlines (see below) and two species (*Pyura stolonifera* and *Pyura herdmani*)
199 that are native but have a sister species that has been reported as highly invasive species
200 somewhere else (Teske *et al.*, 2011) (see sampling sites and general field methodology in
201 Appendix S5). We conducted laboratory experiments under a range of temperatures and
202 measured embryonic development time, and the success of larval development, larval
203 settlement and settler metamorphosis (details of methods in Appendix S6).

204 Given the non-linear nature of rate-temperature relationships (Janion *et al.*, 2010)
205 and the fact that most species' embryos did not develop above 20°C (see Results), we
206 only statistically analysed the linear portion of the reaction norm, i.e. from 10 to 20°C to

207 evaluate interspecific differences. Therefore, we implemented a general linear model with
208 mean embryonic development time as the response variable, and species and temperature
209 as predictors. Interactions between species and temperature indicated differences in
210 reaction norm slopes among species. Given the proportional nature of developmental
211 success data, a generalized linear model using a binomial error structure and a logit link
212 function was used to assess the effects of species, temperature and their interactions on
213 development success. This model was checked for overdispersion and scaled deviances
214 were obtained when necessary. Species differences were determined by examining least
215 squares means and overlap of the Wald 95% confidence limits from the generalized
216 linear model outputs. The same statistical model type was used to assess the effects of
217 species and temperature on the number of successful postmetamorphs at day 3 after
218 fertilization (i.e. those that had completed the larval, settlement and post-metamorphic
219 stages, providing the most complete measure of success). We also investigated the effects
220 of species, temperature and day after fertilization (1^{st} and 3^{rd} day) on the proportion of
221 attached settlers and the proportion of floating (detached from the substratum) settlers in
222 relation to the total number of initial larvae. Finally, we investigated the effects of
223 species, temperature and day after fertilization on the proportion of larvae that failed to
224 settle. All analyses were done with SAS v. 9.1 (SAS Inst., Cary, NC) and Statistica v. 10
225 (StatSoft, Tulsa, USA).

226 **(B)** ***Seawater temperature data***

227 We obtained Sea Surface Temperature recordings from the South African Data
228 Centre for Oceanography (SADCO) (see Appendix S7 and Table S4 for details). We
229 calculated the mean annual temperature, the mean of summer months (January - March),

230 winter months (July - September), and the difference between the annual maximum and
231 minimum temperature recorded each year. Inter-annual trends were tested using linear
232 regression with a critical value of 0.05. In addition, these data provide an estimate of
233 shipping intensity through time, which we calculated by measuring the number of
234 temperature recordings per year. Data were analysed and plotted using R v. 2.10.0 (R
235 Development Core Team, 2011).

236 **(B) *Measuring range and abundance shifts of NIS***

237 To understand recent range shifts of NIS, we combined biogeographic
238 information from taxonomic studies (references in Appendix S3) that included extensive
239 surveys along the South African coast, and our own data. We then plotted the distances
240 among sites where species were recorded to visualize changes in species ranges. We
241 considered five NIS: *Clavelina lepadiformis*, *C. intestinalis*, *A. aspersa*, *S. plicata* and *M.*
242 *squamiger*). All are highly conspicuous and abundant, and are unlikely to have been
243 unnoticed by a specialist. We excluded for this analysis the remaining four NIS obtained
244 in the field surveys (see below). These were two colonial (*Diplosoma listerianum*,
245 *Botryllus schlosseri*) and two solitary NIS (*Ascidia sydneiensis* and *Asterocarpa humilis*).
246 For these, the taxonomy is in debate, so they may contain cryptic species or have been
247 misidentified as closely related species. In order to compare abundance trends, we
248 obtained abundance data for all ascidian species from our field surveys and compared the
249 mean values among status types (native, cryptogenic and NIS) and sampling years (i.e.
250 2007 and 2009).
251
252

253 **(A) RESULTS**

254 **(B) *Field surveys***

255 Combining the results from the sampling in 2007 and 2009, we identified 16
256 species endemic to South African shores, nine NIS that are widely distributed around the
257 world (Fig. S1), and nine species for which the status could not be confirmed and that
258 were included as cryptogenic (Table S5). NIS were the most widespread group, followed
259 by the native species and, finally, the narrowly distributed cryptogenic species (Fig. S2).
260 All NIS were found in at least two biogeographic provinces, with *C. intestinalis* being the
261 most widely distributed species (Fig. 1). In general, ascidian species richness marginally
262 increased eastwards (Fig. S2).

263 **(B) *Comparing regional and global genetic signatures***

264 We obtained a total of 764 COI sequences (Tables S1, S2) with fragment lengths
265 of 546, 786, 639, 655 base pairs (bp) for *C. lepadiformis*, *C. intestinalis*, *S. plicata* and *M.*
266 *squamiger* respectively. *Clavelina lepadiformis* showed the lowest haplotype diversity
267 while *M. squamiger* was the most diverse (Fig. S3, Table S1). Haplotype diversity
268 increased eastwards for *C. lepadiformis* and *C. intestinalis* (Fig. S3, Table S1). When we
269 combined the haplotypes generated in our study with those from GenBank (Table S2), we
270 obtained a final alignment of 366, 692, 560 and 561 bp for *C. lepadiformis*, *C.*
271 *intestinalis*, *S. plicata* and *M. squamiger* respectively. The haplotype networks examined
272 the relationships among haplotypes at a global scale and showed two separate lineages
273 for each species (Fig. 2). These lineages showed different levels of genetic divergence.
274 Firstly, topologies connecting haplotypes with a cumulative probability greater than 95%
275 of being correct were constrained to divergence levels of less than ten and 12 steps for *C.*

intestinalis and *M. squamiger* respectively. Since the number of mutational steps between haplotype pairs did not exceed these values, the two lineages could be connected (Fig. 2). Secondly, the two lineages of *C. lepadiformis* and *S. plicata* exceeded the maximum number of steps, eight and ten steps respectively, and therefore the two lineages could not be connected with 95% probability (Fig. 2). We obtained a total of 15 haplotypes for *C. lepadiformis*, 23 for *C. intestinalis*, 24 for *S. plicata* and 63 for *M. squamiger*. Many haplotypes found in South Africa were also detected elsewhere within the introduced range of the species (Fig. 2). For *C. lepadiformis*, three South African haplotypes were shared with other regions around the world (Fig. 2), of which two had not been sampled before and one was shared with the Azores (Table S2). For *C. intestinalis*, all individuals collected in South Africa were Type A (sensu Nydam & Harrison, 2007). We found eight South African haplotypes that were unique and six that were shared with other regions. For *S. plicata*, South African haplotypes were found across the two global lineages that had been formerly reported (Pineda *et al.*, 2011) with one previously undiscovered South African haplotype found in each lineage (Fig. 3). *M. squamiger* had 15 private haplotypes from South Africa (8 new from our study) and 9 shared with other regions (Fig. 2). In addition, two clear lineages were recovered, of which Lineage 1 contained most haplotypes found in South Africa (Fig 2). The haplotypes obtained from the Azores and Madeira had been previously recorded in other regions (Table S2).

(B) *Effect of temperature on development, settlement and metamorphosis*

Development rate increased slowly up to 20°C, with the slopes of *C. intestinalis* and *A. aspersa* (Fig. S3A) being lower than for the other four species (linear model, species-temperature interaction effects, $F_{(6, 78)} = 296.8$, $P < 0.001$). This difference in

299 slope was mostly due to the development success and relatively long development time
300 of these two species at 10°C. At 25°C, *C. intestinalis*, *S. plicata* and *M. squamiger*
301 showed successful development, while no development occurred for *A. aspersa* or either
302 *Pyura* species (Fig. S4). No species showed larval development at 30°C. Temperature,
303 species and their interaction, all had significant effects on larval development success
304 (Generalized linear model. Temperature, df = 1, Wald Chi-Square = 8.66, $P = 0.003$;
305 Species, df = 6, Wald Chi-Square = 45.46, $P < 0.001$; Temperature x Species interaction,
306 df = 5, Wald Chi-Square = 19.15, $P = 0.002$). The 95% Wald confidence limits for each
307 species indicated that the development success of *A. aspersa* and *C. intestinalis*, and *A.*
308 *aspersa* and *S. plicata* were not significantly different from each other, but estimates for
309 *A. aspersa* and *C. intestinalis* were significantly higher than for *P. stolonifera*, *P.*
310 *herdmani* and *M. squamiger* (Table 2).
311 In the experiment testing the effect of temperature on settlement and
312 metamorphosis, the proportion of successful settlers (defined here as the ones that
313 completed metamorphosis or post-metamorphs) at day 3 was generally highest at higher
314 temperatures (20-25ºC), but the number of total settlers (successful and non-
315 metamorphosed settlers) showed the lowest numbers for most species at the highest
316 temperature (30ºC) (Fig. S5). *P. stolonifera* showed higher settlement success at 20ºC,
317 while other species (e.g. *M. squamiger* and *S. plicata*) performed better at 25ºC. A
318 noteworthy exception was *A. aspersa*, which, at high temperatures (e.g. 25ºC. Fig. S5A)
319 produced settlers despite poor larval developmental success at these temperatures (Fig.
320 S4B). Settlement data showed a significant effect of temperature on all variables
321 analysed: successful settlers, floating settlers and failed larvae (Table 3). When we

322 analysed the effect of each factor and their interactions for each species separately (Table
323 S6), most interactions between the factors Day and Temperature were significant. *Pyura*
324 *spp.* were the only species for which temperature did not have consistent major effects.
325 Floating settlers (i.e. settled to the water surface pellicle, or settlers that started
326 metamorphosis while in the water column) and failed larvae were considered dead. We
327 found an increase of floating settlers with temperature and time, especially at 30ºC for
328 day three, which indicated that temperature stress affected their final success (Fig. S5C).
329 Lower temperatures resulted in the highest proportion of failed larvae, although this trend
330 weakened with time (Fig. S5D). Regarding post-metamorphic stages, we found that most
331 species achieved metamorphosis at three different temperatures, while native species did
332 so at only one or two temperatures (Fig. S6). *A. aspersa* and *C. intestinalis* were able to
333 complete metamorphosis at 15ºC, and only *S. plicata* completed metamorphosis at 30ºC
334 (Fig. S6).

335 The results of the laboratory experiments and resulting temperature tolerance
336 breadth for all stages are summarized in Table 4, and broadly showed that all species'
337 eggs and larvae were able to develop, settle and metamorphose at 20ºC, but only *A.*
338 *aspersa* and *C. intestinalis* were able to do so at 15ºC, and *C. intestinalis*, *M. squamiger*
339 and *S. plicata* at 25ºC.

340 **(B) *Temperature records***

341 The SADCO records indicated that seawater temperature during the period 1960
342 to 2010 has significantly increased in six sites, while remained relatively stable in the
343 remaining five sites (Fig. S7). Most sites showed significant positive slopes in mean
344 winter temperatures, with the exception of the northernmost sites (Alexander Bay and

345 Durban), which lie on opposite coasts. Only four sites showed significant positive
346 regressions for the summer months (Fig. S7). The magnitude of such change in mean
347 values went from + 0.5 to + 1.5 ^{o}C. All sites showed an increasing trend when annual
348 differences between maximum and minimum temperatures were plotted, although only
349 three sites showed significant positive slopes (Fig. S8). At these sites increases in
350 temperature of 2-3 ^{o}C were observed during this period.

351 There was an increase in shipping intensity in the late 1960s and early 1970s,
352 coinciding with the closure of the Suez Canal (Fig. S9). Shipping intensity before and
353 after this period remained relatively consistent with a gradual decline towards the early
354 21st century. Therefore, the contribution of this vector to the spread NIS has not
355 significantly increased over the studied period.

356 **(B)** ***Changes in species ranges and abundance***

357 We found evidence of range expansion among years for NIS (Figs. 3A, S2), while
358 native and cryptogenic species did not vary consistently, with some species showing
359 small range contractions and others expanding (Fig. S2). Among the NIS, *C. intestinalis*
360 was the species that showed the widest range and the greatest range expansion. Observed
361 expansions ranged between *c*. 1000 and 2500 Km (Figs. 3A). NIS were on average more
362 abundant than native and cryptogenic species in both years (Fig. 3B), and increased
363 significantly in abundance between 2009 and 2007 (t-test; t = -2.035, df = 176, P =
364 0.043).

365

366

367

368 **(A) DISCUSSION**

369 We found that NIS are well-established across diverse biogeographic regions in
370 South Africa. In addition, NIS are both expanding their ranges and increasing in
371 abundance, and this trend is independent of species differences in optimal temperature,
372 range size or genetic variability (both at regional and global scales). However, the
373 increase in range and abundance of NIS was synchronized with a trend over the last 50
374 years towards warmer mean temperatures and a wider range of temperatures. This
375 suggests that the facilitative effects of climate change on biological invasions advocated
376 by previous studies at local scales (Stachowicz et al., 2002; Sorte et al., 2010) also occur
377 at regional and perhaps global levels.

378 **(B)** ***Integrating genetic patterns across different spatial scales***

379 Our study revealed two divergent mtDNA lineages with extensive geographical
380 mixing and sympatry of widespread genotypes, which indicates multiple secondary
381 contacts of ancestral lineages at both regional and global scales (see specific details in
382 Appendix S8). Such processes have the potential to generate adaptive differentiation
383 among invasive populations as has been suggested for plant species (Chun *et al.*, 2009).
384 In addition, these patterns could be explained by within-species physiological differences
385 among lineages, and / or limited connectivity among certain harbours. However, this
386 requires further investigation using a more comprehensive dataset (e.g. Rius *et al.*, 2012).
387 When we placed the South African populations within a global context we found an
388 intricate distribution of haplotypes. This suggests a scenario of continuous interchange of
389 propagules due to intense local and international shipping (Kaluza *et al.*, 2010), which
390 has an homogenizing effect on the genetic composition of introduced populations (e.g.

391 Pineda *et al.*, 2011). Thus, the genetic composition of the introduced range most likely
392 consists of a mixture of diverging genotypes from the native range and leading towards a
393 general pattern of global genetic panmixia. The widespread regional and global
394 distribution of these species indicates their adaptation to several climatic regions, and our
395 data suggest that individuals containing certain haplotypes are more widespread and
396 perhaps more adaptable than other individuals of the same species. The range shifts
397 observed for the studied NIS were independent of their global level of genetic diversity.

398 **(B)** ***Effects of temperature on early life-history stages***

399 In the sea the planktonic larval stage has a major influence on enabling dispersal
400 and population connectivity (McQuaid, 2010). The large diversity of evolutionary
401 strategies in the sea has resulted in a wide range of propagule forms. This gives rise to
402 varying degrees of planktonic periods and dispersal capabilities, and can lead to high
403 levels of intraspecific phenotypic plasticity (but see Ling *et al.*, 2008) due to variable
404 conditions. Our experimental results indicate that higher temperature treatments induced
405 earlier settlement and metamorphosis. Some species could not complete egg development
406 at higher temperatures, even though their larvae performed well and completed
407 metamorphosis at these temperatures, which suggests that initial development stages are
408 more sensitive. In contrast, cold or unfavourable environments delayed or constrained
409 larvae settlement and metamorphosis (see also Dybern, 1965; Thiyagarajan & Qian,
410 2003). The trade-off between larval swimming time and range expansion is likely to
411 influence species distributions. When we analysed the influence of artificial transport, our
412 estimates of shipping intensity did not indicate an increasing trend in recent years, which
413 suggests that during the studied period this vector has not increasingly contributed to

414 range expansions. In addition, antifouling practices are becoming more effective, which
415 increasingly limits the transportation of species as stowaways.

416 Our laboratory experiments indicate thermal limitation during early life-history
417 stages, especially at extreme temperatures (10 and 30ºC). This suggests that coastal
418 regions that experience such temperatures during reproductive periods will be unable to
419 support these species. This could explain the absence of some species on the west coast
420 (e.g. *M. squamiger*), where strong upwelling periods can drop temperatures down to 8-
421 9ºC. However, it is known that some groups of organisms can shift their phenology and
422 seasonal thermal tolerance with changing environmental conditions (Millar, 1971; Yang
423 & Rudolf, 2009). Thus, such species are able to adapt or demonstrate plastic responses
424 when facing different thermal conditions or latitudes. Our physiological results showed
425 that the optimal temperature for the studied species were between 15 - 20 ºC, which is in
426 accordance with previous studies (Thiyagarajan & Qian, 2003). However, even if
427 development is assumed to be ideal at such optimal temperatures, suboptimal conditions
428 may also play an important role in species establishment. For instance, although the
429 embryonic development of *C. intestinalis* was improved between 15 and 20 ºC (Fig. S4)
430 (see also Dybern, 1965; Bellas *et al.*, 2003, for performance curves in other regions), this
431 species was capable of settling and completing metamorphosis in both warmer (> 20ºC)
432 and colder conditions (Figs. S5, S6). In general, all NIS showed widespread distributions
433 around the world (Fig. S1), suggesting a broad range of temperature tolerance. The use of
434 thermal tolerance ranges as a predictor of geographic success requires further study,
435 especially when extrapolating physiological outcomes to other regions without

436 accounting for microsite temperature variability (Clusella-Trullas & Chown, 2011) or
437 ecological interactions with the receiver community.

438 **(B) *Thermal tolerance and climate change***

439 In terrestrial ecosystems, temperature has been used to predict both extinctions
440 and the spread of species considering different scenarios of climate change driven by
441 anthropogenic effects (Deutsch *et al.*, 2008). For example, organisms with restricted
442 thermal tolerance have moved to higher elevations and latitudes in response to recent
443 climate change (Angert *et al.*, 2011). For ascidians, temperature exerts a strong influence
444 on reproduction, development, energy requirements and feeding across all life-history
445 stages (Millar, 1971; Thiyagarajan & Qian, 2003). Thus, a slight change in seawater
446 temperature has the potential to affect species survival significantly through lethal and
447 sublethal effects. Warming is believed to have the most deleterious consequences on
448 organisms that are relatively sensitive to temperature change and are currently living in
449 conditions close to their optimal temperature or 'safety margin' (Deutsch *et al.*, 2008).
450 This might be even more critical at the most sensitive ontogenetic stages, for which
451 optimal temperature ranges are narrower (Pineda *et al.*, 2012). Therefore, the biological
452 consequences of rising temperatures depend on the physiological sensitivity of each
453 organism (Somero, 2012) and, as demonstrated here, the accumulative effects through
454 multiple life-history stages. Our results indicate a higher thermal tolerance of NIS during
455 the developmental stages as compared to natives. However, this requires further
456 investigation by including a higher number of phylogenetically dissimilar native species
457 and a broader sampling of conspecifics of different origins to evaluate the role of local
458 adaptation. Empirical evidence is especially needed in aquatic environments, as there is a

459 dearth of studies that analyse the influence of altered environmental conditions on both
460 native and NIS performance in these ecosystems compared to terrestrial ones (Sorte *et*
461 *al.*, 2012).

462 **(B) *Climate change and species invasions***

463 The analysis of ship-based data collected over the last 50 years revealed a
464 significant positive trend at most sites, indicating that temperatures are predominantly
465 increasing. This trend was supported by the annual mean temperature and most especially
466 by the mean temperature of winter months (Fig. S7). Analyses of time-series of infrared
467 satellite imagery suggest a more complex picture, with cooling on the south and south-
468 west coasts of South Africa, with warming on the east coast (Rouault *et al.*, 2010). This is
469 supported by minor, but telling, changes in the distribution of cold-water kelps (Bolton *et*
470 *al.*, 2012). In either event, the situation is one of changing conditions. The differences
471 between maximum and minimum temperature in the ship-based data revealed a positive
472 trend towards more extreme annual temperatures at most sites (Fig. S8). In line with this,
473 extreme climatic events, which are expected to increase in the future, have recently been
474 identified as potential factors enhancing species invasions (Diez *et al.*, 2012). This
475 suggests that species with a wider thermal niche have the potential to benefit from more
476 extreme conditions in the future. Correspondingly, our field data show that biogeographic
477 patterns are related to the thermal-response results obtained during the early life-history
478 stages of the studied species - the most widespread species had greater developmental
479 thermal tolerances, while the lowest and highest experimental temperatures were
480 correlated with the range boundaries of some of the studied species.

481 The warming of seawater temperature has been identified as an important driver
482 of community change (e.g. Sorte *et al.*, 2010). Warmer winter seawater temperatures
483 have been shown to enhance the earlier seasonal arrival of invasive species at local
484 scales, by inducing earlier recruitment (Stachowicz *et al.*, 2002). In contrast, resident
485 species might become increasingly poorly adapted to the local environment, opening
486 colonization opportunities for NIS. Our study provides empirical evidence of range
487 expansions of NIS at multiple spatial scales during a period of change of temperature
488 regimes, which indicates an overall trend towards global biotic homogenization.
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(A) BIOSKETCHES

M.R. is currently a postdoctoral researcher interested in the underlying mechanisms that determine and maintain species ranges and how alterations such as anthropogenic disturbances and biological invasions affect the composition of native assemblages. His research interests include biogeography, population genetics, community ecology and conservation biology, with a special focus on marine foundation species.

718 **(A) SUPPORTING INFORMATION**

719 Additional Supporting Information may be found in the online version of this article.

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740 **Table 1**. Sampled sites included in the present study. The site name abbreviations (Code),
741 the geographic position and the characteristics of each site are indicated.
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Name of the site	Code	Latitude (S)	Longitude (E)	Type
Alexander Bay	AB	28º 46’ 33”	16º 34’ 23”	Oyster farm
Saldanha Bay	SB	33° 00’ 18”	17° 56’ 53”	Small harbour
Table Bay	TB	33º 55’ 22”	18º 26’ 36”	Large harbour
Hout Bay	HB	34º 02’ 60”	18º 20’ 53”	Recreational marina
Mossel Bay	MB	34º 10’ 42”	22º 08’ 40”	Small harbour
Knysna	KA	34º 02’ 29”	23º 02’ 48”	Recreational marina
Port Elizabeth	PE	33º 58’ 02”	25º 38’ 07”	Large harbour
Bushman’s River	BR	33º 40’ 47”	26º 39’ 22”	Recreational marina
Port Alfred	PA	33º 35’ 38”	26º 53’ 31”	Recreational marina
East London	EL	33º 01’ 22”	27º 53’ 45”	Small harbour
Durban	DU	29º 51’ 49”	31º 01’ 23”	Large harbour

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Table 2. Estimates and Wald 95% confidence limits from the generalized linear models testing for the effects of species on larval development success. 'Chi-square' tests whether the estimate is different from zero, alpha is set at 0.05. Significance of pairwise comparisons was determined by non-overlapping Wald 95% confidence intervals.

Species effect	Estimate	SE	DF	Chi-Square	P	Wald 95% confidence limits	
Ascidiella aspersa	-0.1363	0.2730	1	0.25	0.6176	-0.6715	0.3988
Ciona intestinalis	0.5613	0.2789	1	4.05	0.0442	0.0145	1.1080
Styela plicata	-1.2289	0.3306	1	13.82	0.0002	-1.8768	-0.5810
Microcosmus squamiger	-3.8738	0.9614	1	16.23	<.0001	-5.7582	-1.9894
Pyura herdmani	-2.0530	0.2276	1	81.35	<.0001	-2.4991	-1.6069
Pyura stolonifera	-2.1553	0.3492	1	38.11	<.0001	-2.8396	-1.4710

775 **Table 3**. Output of generalized linear models reflecting the effects of species and
776 temperature on: A) overall successful settlers/metamorphs and (B) settlers including
777 incomplete metamorphs. Same models were run including the effects of species,
778 temperature and days on (C) floating settlers and (D) failed larvae. (A) and (B) only
779 include 3rd day after fertilization data whereas (C) and (D) examine the effect of day of
780 observation. Significant results ($P < 0.05$) are indicated in bold.

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Effect	df	Wald Chi-Square	*P*
A) Proportion of successful settlers / complete metamorphs			
Temperature	1	8.43	**<0.01**
Species	6	65.44	**<0.0001**
Temperature x Species	5	4.36	0.50
B) Proportion of settlers including incomplete metamorphs			
Temperature	1	0.09	<0.77
Species	6	71.37	**<0.0001**
Temperature x Species	5	46.88	**<0.0001**
C) Proportion of floating settlers			
Temperature	1	13.49	**<0.001**
Species	6	87.01	**<0.0001**
Days	1	7.19	**0.007**
Temperature x Species	5	26.55	**<0.0001**
Species x Days	5	22.24	**0.0005**
Temperature x Days	1	6.09	**0.01**
Temperature x Species x Days	5	32.74	**<0.0001**
C) Proportion of failed larvae			
Temperature	1	82.33	**<0.0001**
Species	6	126.28	**<0.0001**
Days	1	23.76	**<0.0001**
Temperature x Days	1	31.16	**<0.0001**
Temperature x Species	5	8.91	0.11
Species x Days	5	8.04	0.15
Temperature x Species x Days	5	15.93	**<0.01**

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784 **Table 4**. Summary results of experiments testing the success on egg development (D),
785 larval settlement (S) and settler metamorphosis (M) three days after fertilization at
786 different temperatures for the studied species. ☑ and ☒ indicate success or failure,
787 respectively. Overall failure (indicated in grey) was considered when larval development
788 could not be achieved or when metamorphosis was not completed.

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Species Temperatures	10	15	20	25	30
Developmental stage	D / S / M	D / S / M	D / S / M	D / S / M	D / S / M
Ciona intestinalis	☑ / ☑ / ☒	☑ / ☑ / ☑	☑ / ☑ / ☑	☑ / ☑ / ☑	☒ / ☑ / ☒
Ascidiella aspersa	☑ / ☑ / ☒	☑ / ☑ / ☑	☑ / ☑ / ☑	☒ / ☑ / ☑	☒ / ☑ / ☒
Styela plicata	☒ / ☒ / ☒	☑ / ☑ / ☒	☑ / ☑ / ☑	☑ / ☑ / ☑	☒ / ☑ / ☑
Microcosmus squamiger	☒ / ☑ / ☒	☑ / ☑ / ☒	☑ / ☑ / ☑	☑ / ☑ / ☑	☒ / ☑ / ☒
Pyura herdmani	☒ / ☑ / ☒	☑ / ☑ / ☒	☑ / ☑ / ☑	☒ / ☑ / ☑	☒ / ☑ / ☒
Pyura stolonifera	☒ / ☑ / ☒	☑ / ☑ / ☒	☑ / ☑ / ☑	☒ / ☑ / ☒	☒ / ☑ / ☒

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804 **(A) FIGURE LEGENDS**

805 **Fig. 1**. Map of the Southern African coastline with the sampled sites indicated. Sea
806 surface temperature satellite readings as in 24 June 2007 (Source: magicseaweed.com,
807 NOAA data) are indicated in different colours. The distribution of the studied species
808 (NIS: non-indigenous species, native: native species) found during the 2007 and 2009
809 field surveys is indicated with colour circles. Site abbreviation names and details can be
810 found in Table 1, except RB, which indicates Richard's Bay, where we could not conduct
811 a thorough survey but collected samples of *S. plicata* and *M. squamiger* for genetic
812 analyses.

813 **Fig. 2**. Haplotype network of the species studied (A - *Clavelina lepadiformis*, B - *Ciona*
814 *intestinalis*, C - *Styela plicata* and D - *Microcosmus squamiger*) indicating the presence
815 of each haplotype in: 1. South Africa (in black), 2. The rest of the introduced range (in
816 grey), and 3. Native or cryptogenic ranges (in white). The smaller black circles represent
817 unsampled or extinct haplotypes. Branch sections delimited by two circles indicate single
818 mutational steps, irrespective of their branch length.

819 *Footnote*: *Clavelina lepadiformis* - Lineage 1 is the Atlantic clade and Lineage 2 is the
820 Mediterranean clade of Turon *et al*. (2003); *Styela plicata* - Lineage 1 is group 1 and
821 Lineage 2 is group 2 of Pineda *et al*. (2011); *Microcosmus squamiger* - Lineage 1 is
822 group H1 and Lineage 2 is group H2 of Rius *et al*. (2008).

823 **Fig. 3**. Changes in species ranges and abundance along the South African coast. A)
824 Range expansion estimates of the studied non-indigenous species. We included data from
825 taxonomic records (1950, 1960 and 2000) and our surveys (2007 and 2009) to calculate
826 the distance between the most distant sites where each species has been documented. B)

827 Comparison of mean relative abundance between sampled years of native, cryptogenic
828 and non-indigenous species (NIS), pooling data from all sites and species.
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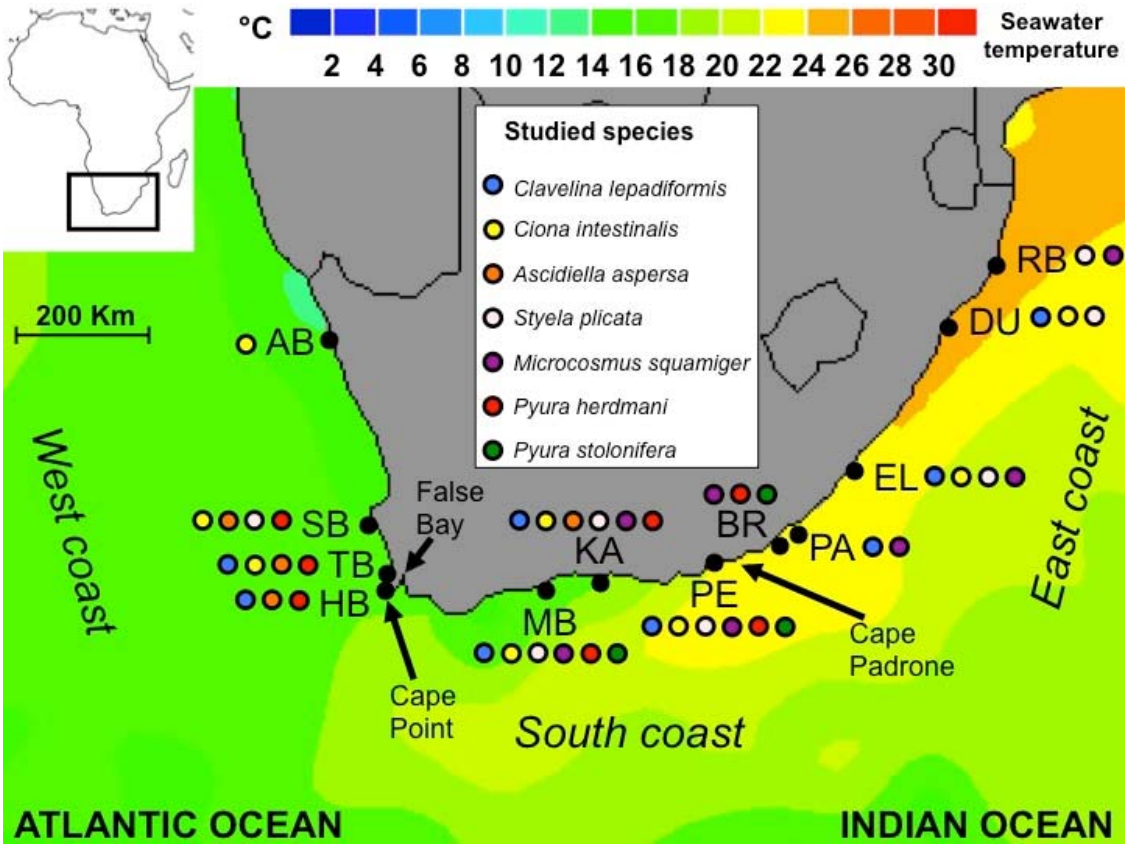
Fig. 1


°C
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Seawater temperature
Studied species
Clavelina lepadiformis
Ciona intestinalis
Ascidiella aspersa
Styela plicata
Microcosmus squamiger
Pyura herdmani
Pyura stolonifera
200 Km
AB
SB
TB
HB
False Bay
KA
MB
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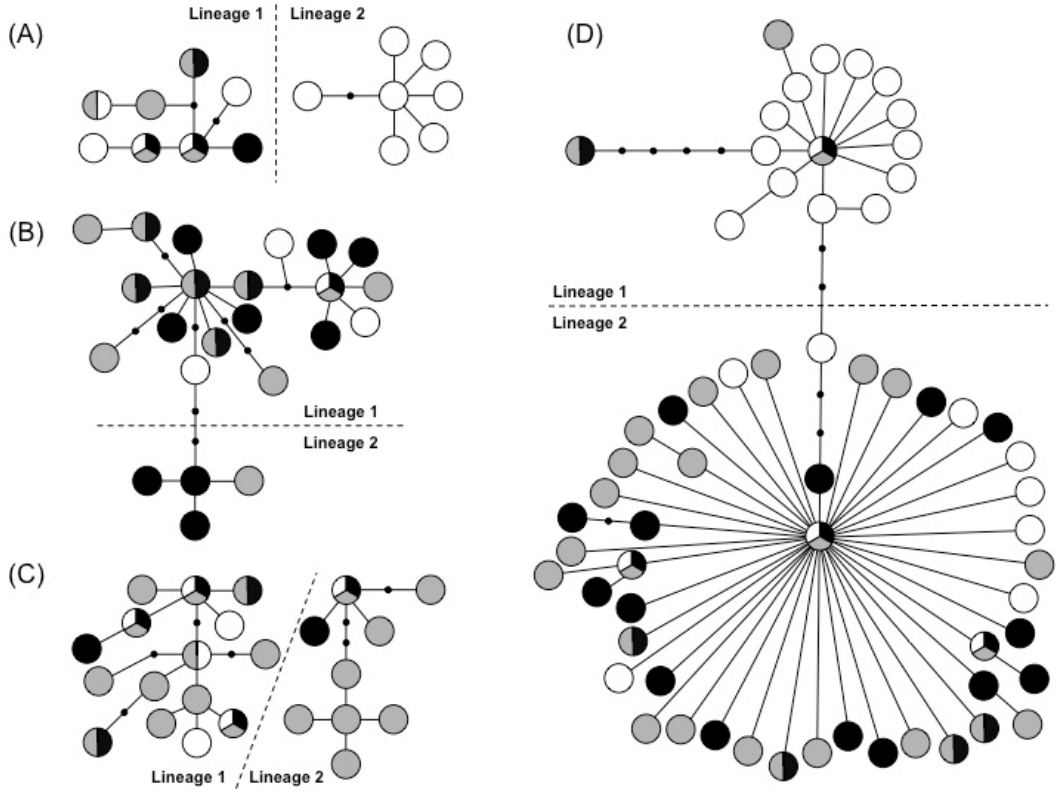
South coast
ATLANTIC OCEAN
INDIAN OCEAN

Fig. 2


(A)
Lineage 1
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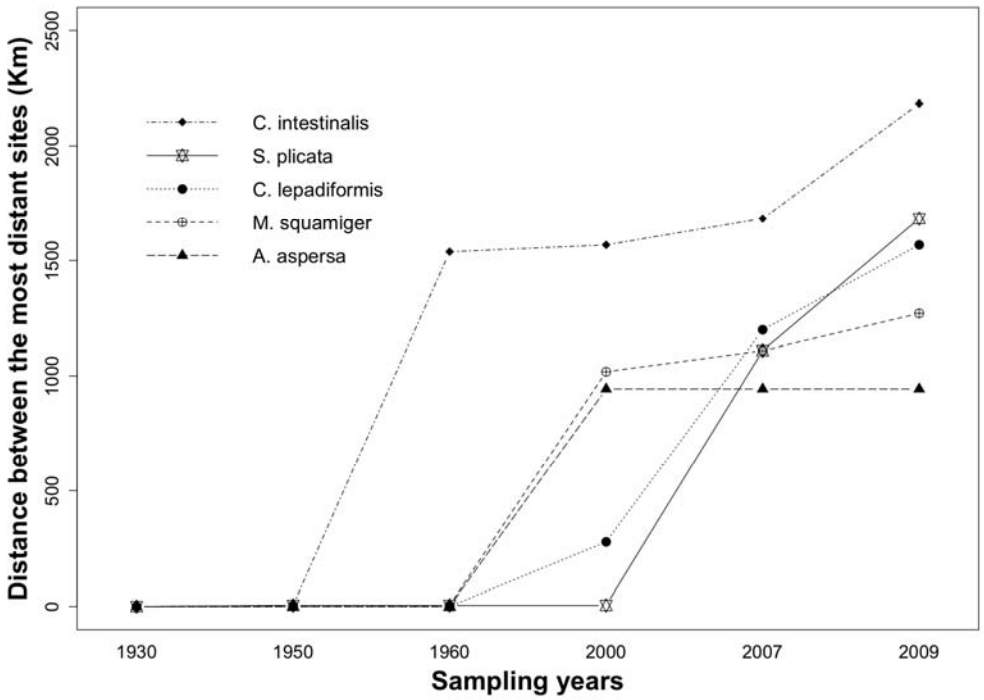
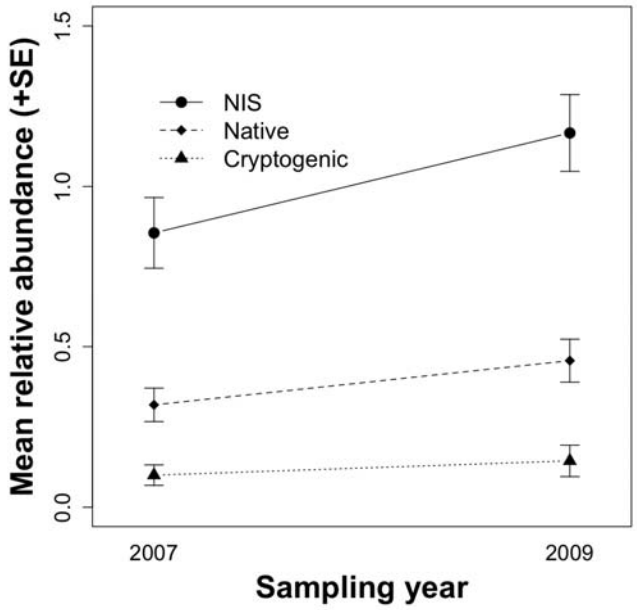
Lineage 2
(C)
Lineage 1
Lineage 2
(D)
Lineage 1
Lineage 2

Fig. 3.

A)


Distance between the most distant sites (Km)
0
500
1000
1500
2000
2500
C. intestinalis
S. plicata
C. lepadiformis
M. squamiger
A. aspersa
1930
1950
1960
2000
2007
2009
Sampling years

B)


Mean relative abundance (+SE)
0.0
0.5
1.0
1.5
NIS
Native
Cryptogenic
2007
2009
Sampling year