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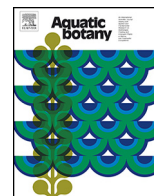
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# Effects of temperature, salinity and seed age on induction of *Zostera japonica* germination in North America, USA



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## ABSTRACT

Seagrasses can colonize unstructured mudflats either through clonal growth or seed germination and survival. *Zostera japonica* is an introduced seagrass in North America that has rapidly colonized mudflats along the Pacific Coast, leading to active management of the species. Growth and physiology have been evaluated; however, there is little information about the factors influencing seed germination. We examined the effects of storage and induction temperature (10, 15, 20 °C) and salinity (0, 10, 20, 30), and storage period (1.5 and 26 months) on germination of seeds of the seagrass *Z. japonica* collected from Yaquina Bay, Oregon, USA. Seed germination at 15 and 20 °C was 1.24 times higher than at 10 °C. Cumulative seed germination at salinity 0 during the first 28 days was 6.5 times greater than at a salinity of 10; similarly, initial seed germination at a salinity of 10 was 7.3 times greater than that observed for salinity 20 and 30. The proportion of germinated seeds collected in 2011 and stored for 26 months was 1.24 times greater than seeds collected in 2013 that were stored for only 6 weeks. Overall average germination rates were 21.6% and 17.1% for 2011 and 2013, respectively. Our experimental results indicate that salinity had a much stronger control over *Z. japonica* germination than temperature, and the long storage period suggests that *Z. japonica* is capable of developing a persistent seed bank. We hypothesize that *Z. japonica* uses seasonal variations in temperature and salinity to avoid competition between generations favoring germination under conditions that are not optimal for the growth of mature plants.

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## 1. Introduction

Seagrass beds are often considered to be primarily clonal; however, recent work indicates that many species have greater genetic diversity than would be expected based on clonal growth (Ruckelshaus, 1996; Reusch et al., 2000; Procaccini et al., 2007; Hughes and Stachowicz, 2009), highlighting the importance of sexual reproduction. Recent work has also highlighted the underappreciation of seagrass “movement ecology” including the role of animal vectors, seed dispersal, clones, etc. (Kendrick et al., 2012; McMahon et al., 2014). Likewise, increased genetic variation has been linked with increased population resilience and vigor (Hughes and Stachowicz, 2004; Ehlers et al., 2008; Reynolds et al., 2012). Despite the potential importance of seeds to the growth and maintenance of seagrass populations and genetic diversity, relatively little work has focused on factors influencing seed banks, dormancy

and germination (Baskin and Baskin, 1998; Orth et al., 2000, 2006; Jarvis et al., 2014).

In theoretical plant population ecology, successful seed germination has been described by analogy as the result of passing through an “environmental sieve” to reach a “safe site” where, appropriate stimuli and resources trigger germination (Harper, 1977). Seed dormancy can be viewed as an adaptation that gives a seed time to pass through the environmental sieve and reach a safe site (Harper, 1977). Likewise, it is fairly well established that differences between what is beneficial for the parent and what is beneficial for the offspring lead to “parent-offspring conflict” (sensu Trivers, 1974). The specific conditions required to break dormancy (e.g., stratification and stimulation of germination) can be viewed as a mechanism to minimize competitive interactions (e.g., conflict) between successive generations (Ellner, 1986; Schupp, 1995; Starrfelt and Kokko, 2010). For marine angiosperms, relatively little is known about either the stimuli that trigger seed germination or the mechanisms used to minimize parent-offspring competitive interactions.

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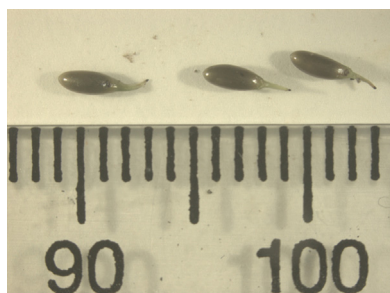


Fig. 1. Mature fruits of *Z. japonica* each containing a seed (scale in mm).

In just over 50 years since the discovery of the introduced seagrass *Zostera japonica* in Willapa Bay, WA, this species has spread and is now established from Johnstone Strait and Vancouver Island in Canada, to Humboldt Bay in northern California (Wyllie-Echeverria and Ackerman, 2003; Shafer et al., 2014). *Z. japonica* is believed to have been unintentionally introduced with aquaculture products, and occupies previously unvegetated intertidal habitat; likely mechanisms of propagule transport include avian and human vectors (see reviews by Shafer et al., 2014; Mach et al., 2014). Recent research has concluded that *Z. japonica* is eurythermal with optimal growth at 20 °C (Shafer et al., 2008) and a lethal threshold at 35 °C (Kaldy and Shafer, 2012). North American *Z. japonica* populations have optimal growth at salinity 20 but are euryhaline, even tolerating daily freshwater immersion (Kaldy, 2006; Shafer et al., 2011). Based on laboratory studies, vertical zonation patterns are controlled by temperature not light (Shafer and Kaldy, 2014; Kaldy et al., 2015). However, factors controlling reproductive biology and colonization success are poorly characterized. Understanding the reproductive biology of this non-native seagrass that is rapidly colonizing unstructured (e.g., unvegetated) mudflat is critical to developing appropriate management strategies based on ecological effects (Mach et al., 2014).

The frequency and intensity of reproductive effort varies among *Z. japonica* populations from north to south within the species' range in North America. Near the northern limits of its range in British Columbia, *Z. japonica* is considered to be an annual or short-lived perennial and rarely over-winters; new populations are initiated each year from seed produced the previous year (Harrison, 1979, 1982a, 1982b; Harrison and Bigley, 1982). In Canada, flowering shoots begin to appear in May, with peak flowering occurring in late July and August (Harrison, 1979; Bigley, 1981; Harrison, 1982a, 1982b; Ruesink et al., 2010). In Padilla Bay, Washington, *Z. japonica* flowering shoots were observed in late June (Shafer, 2007). A Yaquina Bay, OR population of *Z. japonica* was perennial, persisting throughout the year; flowering began in July, reached a peak in September–October, and continued into December (Kaldy, 2006). Recent work concluded that disturbance can increase *Z. japonica* flowering (Park et al., 2011).

*Z. japonica* inflorescences consist of a one-sided spadix with 4–7 female flowers and 4–5 male flowers (den Hartog, 1970; Bigley, 1981). Seeds develop from ovules produced within an ovary, which when mature becomes the fruit. In many seagrasses, both seed and fruit are closely associated, comprising a single entity at the time of dispersal. *Z. japonica* fruits (Fig. 1) are reddish-brown in color, with a thin pericarp (den Hartog, 1970). Seeds are elliptical, ranging between 1.9 and 2.6 mm in length (Wyllie-Echeverria et al., 2006); the testa is dark brown, smooth, and shiny with fine striations (Bigley, 1981).

Although, seeds are likely to play an important role in the establishment and spread of *Z. japonica* along the Pacific coast of North America, relatively little is known about the factors that control germination or seedling establishment. The climate of Pacific

Northwest coastal estuaries is characterized as Mediterranean, with warm, dry summers and cool wet winters (Emmett et al., 2000; Lee and Brown 2009). This climate, coupled with *Z. japonica* colonization at high elevation in the intertidal zone (+1 to +2.3 m MLLW) where they are periodically exposed to air during low tide, results in plants that are subject to extreme thermal and salinity variations. We postulate that winter temperatures provide cold stratification and subsequent precipitation induced low salinity stimulates germination while the return to marine salinity favors seedling development. Consequently, temperature and salinity cues may be important triggers for *Z. japonica* seed germination. Although *Z. japonica* seed germination in Japan is known to be strongly influenced by cold stratification (e.g., artificial cold exposure to mimic winter) and induction temperature (Abe et al., 2009; Morita et al., 2011), those experiments did not address salinity effects. Additionally, reviews highlighting factors known to influence seagrass seed germination call for further evaluation of the role of salinity in breaking dormancy (Orth et al., 2000, 2006). Therefore, we were specifically interested in the roles of temperature, salinity and their interaction in regulating *Z. japonica* seed germination. We tested the role of seasonal exposure to low salinity conditions in stimulating *Z. japonica* seed germination, which may reduce inter-generational competition. Since seagrasses in the genus *Zostera* are generally believed to have a transient (<1 year) rather than a persistent (>1 year) seed bank (Orth et al., 2000, 2006; Jarvis et al., 2014), we also evaluated the potential longevity of *Z. japonica* seeds in storage as a proxy for potential seed bank longevity. To complete this work, we used previously established protocols optimized for aquatic plants to assess seed germination success (Ailstock et al., 2010).

## 2. Methods

### 2.1. Seed collection and processing

Protocols for seed collection, processing, storage, and statistical analysis were similar to those described in Ailstock et al. (2010). In August 2011 and September 2013, reproductive and vegetative *Z. japonica* leaf material was collected by hand from the Sally's Bend portion of the Yaquina Estuary in Newport, OR (44.627 N Lat., 124.013 W Lon.), leaving the rhizome and root structures in place. Environmental characteristics were similar to previous descriptions (Kaldy, 2006; Lee and Brown, 2009). No attempt was made to separate reproductive shoots (with flowers and seeds) from vegetative material. Reproductive structures in the collected leaf material occurred in various stages of development and ranged from immature flowers to stalks from which mature fruits had already detached. Leafy material was placed in coolers and transported to the lab for processing within 2 h of collection.

Processing was defined as the method of isolating the mature fruits from the stems and other less developed reproductive structures (Raghavan, 2000; Benech-Arnold and Sanchez, 2004). In the lab, plants were rinsed with seawater over a 1 mm sieve to remove excess sediment and epiphytes. Plants were then placed into two tanks (90 cm × 60 cm × 60 cm) with flowing seawater from Yaquina Bay and aerated vigorously to prevent anoxia. Saturating light levels were provided to plants in order to support developing embryos with maternal photosynthate. Photosynthetically active radiation (PAR; 400–700 nm λ) was measured using a LI-1400 datalogger and LI-192 cosine corrected underwater sensor (LI-COR, Lincoln, Nebraska, USA). In 2011, PAR was supplied by 1000 W metal halide lamps (400 μmol photons m<sup>-2</sup> s<sup>-1</sup>), while in 2013 PAR was provided by LED lights (~200 μmol photons m<sup>-2</sup> s<sup>-1</sup>). Each LED panel contained 119 diodes in a 6:1:1:1 ratio of red (660 nm λ), blue (465 nm λ), orange (620 nm λ), and white

(6000 k) diodes. *Z. japonica* is photosynthetically saturated at about 80–160  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  with no evidence of photo-inhibition at irradiance up to 800  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Shafer and Kaldy, 2014). The light:dark cycle was 12:12 in both years. Seeds were allowed to mature and settle to the bottom of the tanks for 2–3 weeks. Floating leaf material was removed and discarded, while seeds and accumulated detritus were siphoned out of the tank and retained. Siphoned materials were passed over a 4 mm sieve to remove large debris, while seeds and detrital material retained on a 0.5 mm sieve were held for further processing. Fine detrital material was separated from the *Z. japonica* seeds by “winnowing”. Water was added to the seed slurry in a 1 l beaker, agitated and the dislodged detritus was poured off with the supernatant. This process was repeated until only seeds remained. Subsequently, seeds were held in flow through seawater with vigorous aeration until shipment. *Z. japonica* seeds were shipped cold via overnight courier from Oregon to the laboratory in Maryland.

All seeds were stored in the dark at 4 °C at a salinity of 34 (Hawaiian Marine Salts, Houston, Texas) without aeration until initiation of germination tests. Volume and salinity were adjusted monthly as needed, but there was no complete water exchange during storage. Seeds collected in 2011 were stored for a period of 26 months prior to testing; prematurely germinated and rotten seeds were culled prior to germination testing. Seeds collected in 2013 were stored for a period of 6 weeks prior to testing. For the 2011 seed collection, seed germination in storage was assessed at intervals of 6 and 14 months.

## 2.2. Germination tests

Germination tests were initiated in November 2013. Germination was explicitly defined as the emergence of the cotyledon from the seed coat; while dead/rotten seeds were characterized as soft and yielding to pressure. Seeds were removed from storage and placed in plastic Petri dishes containing 30 ml of de-ionized water adjusted to initial test salinities (0, 10, 20, and 30). To examine the effect of induction temperature on seed germination, replicate plates were incubated in water adjusted to test salinities as described above at temperatures of 10, 15, and 20 °C. Five replicate Petri dishes containing 50 seeds each ( $n = 250$  seeds) were exposed to each combination of induction salinity and temperature for a period of 28 days. Induction temperatures were selected to simulate the range of temperatures observed during the time of release from the plants (Kaldy, 2006). Petri dishes were incubated on a 12:12 light:dark cycle at 70  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  photosynthetically active radiation (PAR). The number of germinated *Z. japonica* seeds in each replicate dish was counted at intervals of 7, 14, 21, and 28 days. This will be referred to as the initial germination period.

At the end of the initial germination period (day 28), all decayed and germinated seeds were removed, and the number of remaining seeds in each replicate Petri dish was counted. The liquid in each Petri dish was removed, seeds were blotted dry, and placed in clean dishes containing 30 ml of de-ionized water. Plates were incubated under the conditions described previously. The number of germinated *Z. japonica* seeds in each replicate dish was counted after 35, 42, 49, and 56 days. This will be referred to as the secondary germination period. The secondary germination period was used to evaluate germination in response to the simulation of winter field conditions associated with precipitation events and reduced salinity.

## 2.3. Data analysis

The number of germinating seeds in each treatment represents count data, which obey the Poisson distribution (Freund and Wilson, 1993). For the Poisson distribution, the mean and the

variance are equal; hence, as the mean increases so does the variance, thus violating the assumption of homogeneity of variances required for traditional linear models (Zar, 1996). Poisson regression techniques are well-suited for the analysis of relationships between an observed count (e.g., numbers of germinating seeds) and a set of explanatory variables (Koch et al., 1986). If the total initial number of seeds is  $N$ , the mean germination rate can be modeled as:  $\log(\mu/N) = \alpha + \chi\beta$ , which can be rearranged as  $\mu = N e^{\alpha} e^{\chi\beta}$ .

Poisson regression modeling techniques were applied to establish the relationships between cumulative germination of *Z. japonica* seeds over time and various parameters, (e.g., collection date, induction temperature and salinity). The same technique was also applied to examine the effect of treatment conditions on the numbers of ungerminated (rotten) seeds. If the main effects were significant, linear contrasts and incidence ratios were used to provide estimates of the differences in seed germination between levels of the main effects. Incidence ratios are estimated by exponentiating the coefficients of the Poisson model or by exponentiating the value of linear contrasts of the main-effects coefficients in the effects model (Stokes et al., 2000).

## 3. Results

### 3.1. Seed germination in storage

After 6 months of cold (4 °C) storage in the dark at a salinity of 34, germination of *Z. japonica* seeds collected in 2011 was relatively low (9%). However, after 14 months of continuous exposure to the storage conditions, more than half (52%) of the seeds had germinated; suggesting that a portion of the seed appears to be capable of germinating under low temperature and high salinity conditions. Germinated seeds were discarded, and the remaining un-germinated seeds were used for subsequent germination tests. Germination of seeds collected in 2013 was not noted during the 6 week storage period prior to testing.

### 3.2. Initial seed germination (day 1–28)

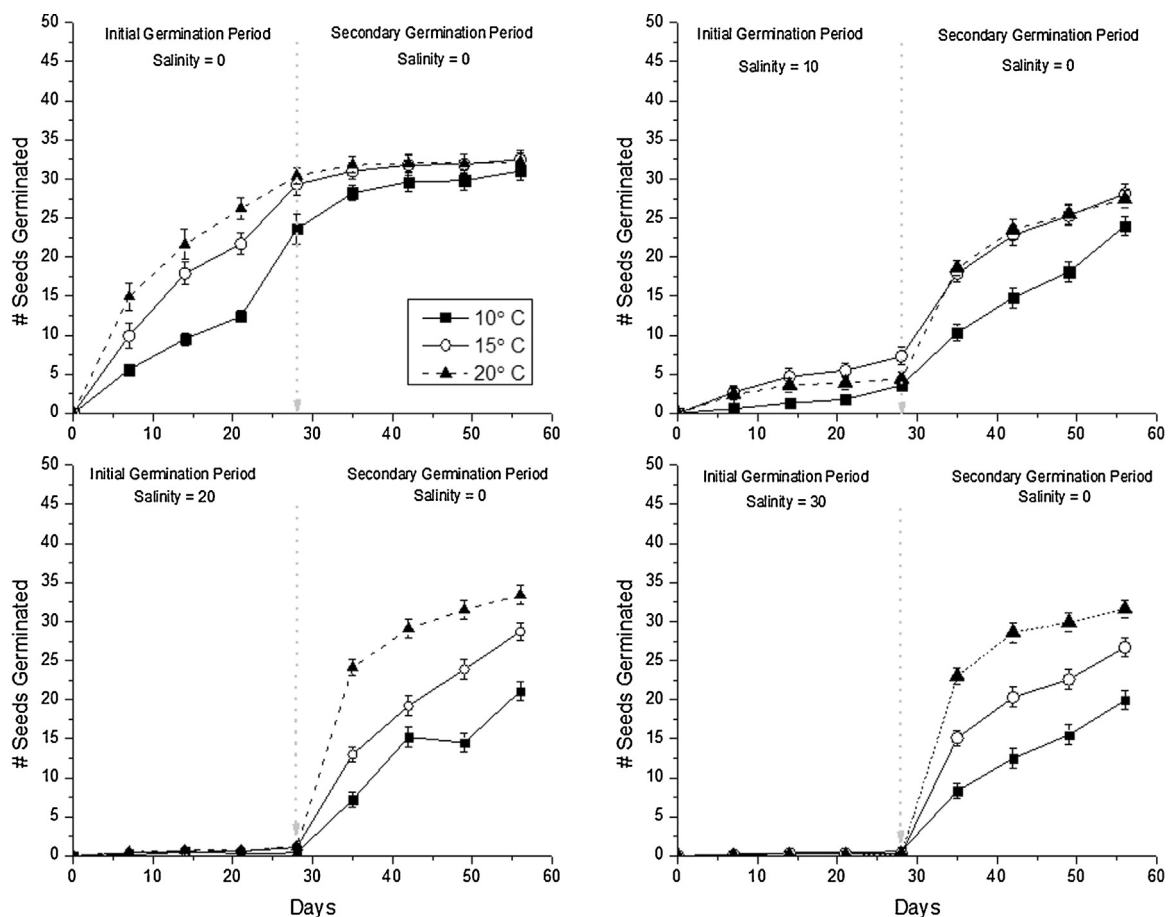
The Poisson regression model predicting the cumulative number of *Z. japonica* seeds germinating as a function of collection date was not significant (Table 1), indicating that there were no differences in the germination of seeds collected in 2011 and 2013. Because year was a blocking factor, the model effects can be interpreted directly.

**Table 1**

Results of the poisson regression analysis of *Z. japonica* seed germination and incidence of rotten seeds as a function of collection year, induction temperature and salinity.

Effect tests	DF	Chi-square	Probability > chi-square
Initial germination period (days 1–28)			
Year	1	2.067	0.151
Temperature	2	9.844	<b>0.007</b>
Salinity	3	469.370	<b>&lt;0.0001</b>
Temperature × salinity	6	14.830	<b>0.022</b>
Secondary germination period (days 28–56)			
Year	1	30.513	<b>&lt;0.0001</b>
Temperature	2	39.888	<b>0.0002</b>
Salinity	3	85.790	0.0885
Temperature × salinity	6	67.085	<b>&lt;0.0001</b>
Incidence of rotten seeds after 28 days			
Year	1	19.468	<b>&lt;0.0001</b>
Temperature	2	2.434	0.2962
Salinity	3	9.884	<b>0.0196</b>
Temperature × salinity	6	14.028	<b>0.0293</b>





**Fig. 2.** Mean cumulative numbers of *Z. japonica* seeds germinating as a function of induction salinity and temperature. Fruits were harvested in the summer of 2011 and 2013 and stored at 4 °C at a salinity of 34 prior to testing. Germination tests consisted of 5 replicates of 50 mature fruits each for each treatment combination, incubated for 8 weeks under a 12 h:12 h light:dark cycle ( $70 \mu\text{mol}/\text{m}^2/\text{s}^{-1}$  photosynthetically active radiation (PAR)). Error bars indicate standard error of the mean.

The data analysis further indicates that there were significant differences observed between temperature levels, salinity levels and their interaction term (Table 1). Examination of the interaction profiles revealed a pattern of orderly interactions, e.g., the means for the levels on one factor remain the same even though the magnitude of the differences between the level of the second factor may change from level to level of the first factor (Ott and Longnecker, 2001). Ott and Longnecker (2001) state “When the interaction is orderly, a test on main effects can be meaningful; a disorderly interaction can obscure the main effects.” Hence, in this case, the main effects can be interpreted; the main effects of both temperature and salinity were significant (Table 1). The proportion of seeds that germinated was higher at 15 and 20 °C than at 10 °C (Fig. 2). The average percent germination for the three temperature levels was 14.1%, 19%, and 18.1%, for 10, 15, and 20 °C, respectively. The incidence ratios computed from the contrasts indicate that seed germination during the first 28 days at 15 °C was 1.24 times greater than at 10 °C; there were no differences in seed germination between 15° and 20 °C, when all other factors were held constant.

The number of germinated seeds decreased with increasing salinity (Fig. 2). The average germination rates were 55.3%, 10.2%, 1.8%, and 0.73%, for salinity levels of 0, 10, 20, and 30, respectively. The incidence ratios computed from the contrasts indicate that cumulative seed germination at salinity 0 during the first 28 days was 6.5 times greater than at a salinity of 10; similarly, initial seed germination at a salinity of 10 was 7.3 times greater than that observed for the average of the 20 and 30 salinity levels.

The number of rotten seeds found at the end of day 28 was significantly different depending on the year in which they were collected (Fig. 3; Table 1). Seeds collected in 2011 had significantly fewer rotten seeds compared to the seeds collected in 2013; likely because seeds that had rotted or prematurely germinated during storage were removed prior to germination tests. Because the interaction term was disorderly, a series of contrasts were performed to compare treatments. At all temperatures, the number of rotten seeds observed after day 28 was significantly larger in freshwater treatments than in the non-freshwater (salinity) treatments (Fig. 3). At 10 °C, the number of rotten seeds in freshwater was 1.95 times greater than that observed at salinity 10, 20 and 30, which were similar. The same pattern was observed for the 15 °C treatment level; at 15 °C the number of rotten seeds observed in the freshwater treatment group was 1.91 times greater than that observed in the salinity treatment groups (Fig. 3). At 20 °C, not only did the freshwater group display the largest number of rotten seeds, but there were significantly greater numbers of rotten seeds in the salinity 10 treatment than in the salinity 20 treatment. At all temperatures, there was insufficient evidence to detect differences in the number of rotten seeds after day 28 between the 20 and 30 salinity treatments (Fig. 3).

### 3.3. Secondary seed germination (day 28–56)

In contrast to the first germination period, the effect of year was highly significant during the second germination period (Table 1). Because year was a blocking factor, the model effects can be interpreted directly. The average germination rates were 21.6% and

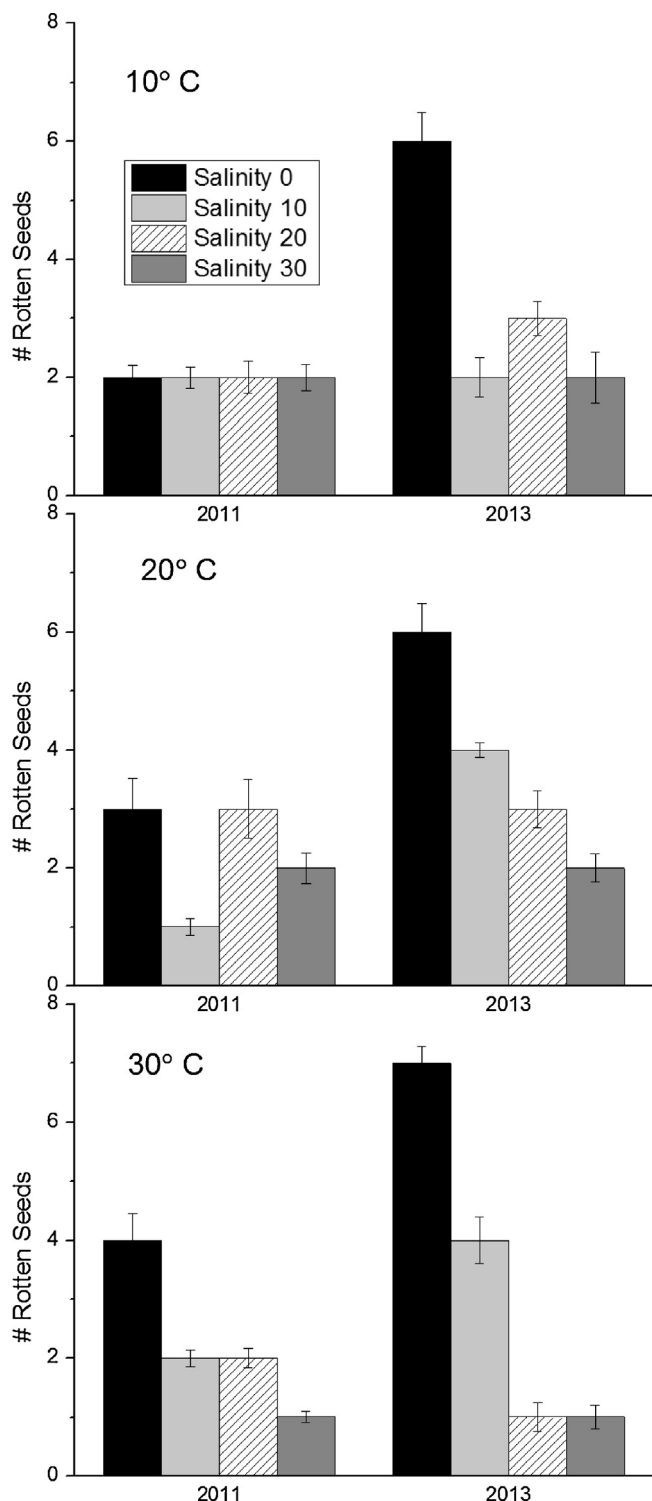


Fig. 3. Mean ( $\pm$  se) dead/rotten *Z. marina* seed counts as a function of temperature and salinity treatments after 28 d.

17.1% for 2011 and 2013, respectively. The proportion of germinated seeds collected in 2011 and stored for 26 months was 1.24 times greater than seeds collected in 2013 that were stored for only 6 weeks.

During the secondary germination period (day 28–56), when all seed lots were exposed to fresh water, there was little increase in the total number of germinated seeds in the initial freshwater treatment (Fig. 2). However, there were dramatic increases

in the number of germinated seeds in those that had previously been exposed to higher salinity conditions (Fig. 2). Due to the non-orderly nature of the highly significant temperature and salinity interaction effect (Table 1), the main effects were not interpreted and a series of linear contrasts were used to compare treatments. Germination of seeds that were exposed to fresh water for the first 28 days was significantly lower than seeds that had been placed in salinity 10, regardless of temperature. At 15° and 20°C, seeds that were exposed to salinity 20 for the first 28 days exhibited 30–40% higher germination than seeds that had been exposed to salinity 10. For all temperatures, there were no differences in seed germination between seed lots that were exposed to salinities of 20 and 30 for the first 28 days.

#### 4. Discussion

Although the germination ecology of a few seagrass species has been studied, it appears that about half of them have seeds that are not dormant at maturity (Baskin and Baskin, 1998; Orth et al., 2000). Of those seagrasses whose seeds exhibit some form of dormancy, most are thought to be physiological dormant (Baskin and Baskin, 1998; Orth et al., 2000, 2006). Physiological dormancy in *Zostera marina* may be broken by warm or cold stratification treatments, depending on where the seeds were collected (Baskin and Baskin, 1998; Orth et al., 2000). In contrast, *Zostera noltii* does not appear to exhibit seed dormancy (Alexandre et al., 2006). Most mature seeds of *Z. japonica* appear to have a physiological dormancy which may be overcome by cold stratification under normal in situ conditions. In British Columbia, *Z. japonica* seedlings appear in March (Bigley, 1981; Harrison, 1982b). Freshly collected *Z. japonica* seeds from Japan exhibited low germination rates when placed in seawater at 10–23°C without cold pre-treatment; subsequent exposure to fluctuating temperatures stimulated germination (Abe et al., 2009; Morita et al., 2011). However, a small proportion of the seeds may be non-dormant when shed and are capable of germinating in the absence of cold stratification, particularly under low salinity conditions (Morita et al., 2011).

We observed fairly high levels of *Z. japonica* seed germination (up to 60%) under experimental conditions, which compares well with field observations indicating that a maximum of 45–57% of new shoots develop from seed (Ruesink et al., 2010). Germination of *Z. japonica* seed was inhibited at salinities of 20 and above, but germination resumed when seeds were placed in distilled water (Bigley, 1981; this study). Stimulation of seed germination by fresh or low salinity water is well known for a variety of *Zostera* species (Hootsmans et al., 1987; Orth et al., 2000). The effect of salinity on seed germination may also be influenced by the seed's dormancy state at the time of testing. For example, cold stratified seeds of *Z. marina* show increased ability to germinate at high salinities (Hootsmans et al., 1987; Harrison, 1991), which may explain the high rates of premature germination in storage for seeds collected in 2011 observed in this study. In contrast to some terrestrial seeds which are stimulated by red light, the exposure of plants and seeds to red dominated light field in 2013 did not result in premature germination.

Our results suggest that seasonal pulses of cold temperatures coupled with low salinity may stimulate *Z. japonica* seed germination. This conclusion is supported by a recent 2 year study that reported finding *Z. japonica* seedlings only during the winter wet season at six sites in Yaquina Bay, OR, USA (Henderson, 2014). However, prolonged exposure to freshwater is likely to result in increased incidence of rotten seeds and poor subsequent seedling survival. Consequently, it would appear that optimum conditions for *Z. japonica* seed germination consist of cold temperature stratification with brief pulses of low salinity (<10) conditions followed by

a gradual return to higher salinity conditions that favor the development of seedlings and adult plants. This is similar to estuarine salinity dynamics following significant storm events during the wet season in Oregon coastal systems. Because *Z. japonica* occupies the high intertidal (Shafer et al., 2014), precipitation falls directly on *Z. japonica* beds exposed at low tide during the winter wet season (J. Kaldy personal observation). Flooding from storm events in the watershed can reduce salinity in Yaquina Bay to near zero and have been shown to influence oyster survival (Burt and McAlister, 1959). Loques et al. (1990) also observed that although germination of *Z. noltii* seeds was greatest at a salinity of 1, the highest initial survival was exhibited by those germlings that germinated and developed at a salinity of 10. We suggest that the combination of cold stratification combined with the low salinity germination stimulation effectively acts as an indicator that a “safe site” for germination has been reached after passing through an “environmental sieve” (sensu Harper, 1977; Orth et al., 2003). The subsequent return to higher salinity conditions would likely favor establishment and maturation of recently germinated seedlings.

The contrasting salinity conditions that favor seed germination and subsequent development of the seedling to the adult stage suggests that these plants may exploit seasonal variations to minimize competitive interactions (e.g., conflict) between successive generations. Plant population ecologists recognize the role of parent-offspring conflict as an important force shaping the recruitment of new individuals to populations (Uma Shaanker et al., 1988). These conflicts imply that there are differences between what is beneficial to a parent and what is beneficial to an offspring (Uma Shaanker et al., 1988; Schupp, 1995). Within-individual conflicts can also occur when conditions that favor one life history stage are disadvantageous for another stage; however, this concept has received little attention (Schupp, 1995). Patterns of differential patch or environmental suitability can be described as a continuum from fully concordant to fully discordant. Fully discordant describes the case where the environment most suitable for the seed is the least suitable for seedling success (Schupp, 1995). The greater the extent of discordance, the greater the level of seed-seedling conflict. We suggest that the contrasting salinity conditions that favor *Z. japonica* seed germination and subsequent development of the seedling to the adult stage is an example of discordant habitat suitability and that it effectively minimizes parent-offspring conflict (Ellner, 1986; Schupp, 1995; Starrfelt and Kokko, 2010). Further research is recommended to determine the conditions that promote optimum survival and development of seedling stages of *Z. japonica*.

The results of this study have important implications for resource managers in North America who are engaged in programs to control or remove this non-native species. Seagrasses in the genus *Zostera* are generally thought to have a transient (<1 year) rather than a persistent (>1 year) seed bank (Orth et al., 2000; Jarvis and Moore, 2010; Jarvis et al., 2014). However, *Z. noltii* in Germany has been shown to have a persistent seed bank for at least 2–3 years (Zipperle et al., 2009). Recent work demonstrated that under laboratory storage conditions, about 30 % of *Z. marina* seeds remained viable after 3 years of storage as determined by vital staining methods, but only 15% of seeds actually germinated after this extended storage period (Dooley et al., 2013). The results presented here indicate that *Z. japonica* seeds can remain viable in storage for at least 26 months, suggesting that they may be capable of developing a persistent seed bank in situ. This inference is supported by Bigley (1981) who found that 75% of “old seed” (obtained from the top 20 cm of sediment) was still viable after almost a year in storage at 5 °C and salinity 27. Additionally, he noted that seed bank *Z. japonica* germination primarily occurred at sediment depths of 4–7 cm during the March–June time frame. If *Z. japonica* is capable of developing a persistent seed bank in situ, then management strategies

aimed at control or removal of existing populations may need to be continued for several years to prevent re-establishment of new populations from existing seed banks.

We theorize that, there may be several patterns in the genetic structure of *Z. japonica* in North America. First, we would expect genetic analysis to reveal high levels of diversity within a population given the apparently heavy reliance on seed production (Kendrick et al., 2012). Although Kaldy (2006) documented low flowering rates in Yaquina Bay, this may have been biased by limited spatial sampling or interannual variability; more recent work appears to suggest larger reproductive events at some sites within the Yaquina estuary (Henderson, 2014). Secondly, if latitudinal patterns in the proportion of flowering hold true (e.g., annual populations in BC, perennial, low flowering in Oregon) then we might expect a North–South gradient in the degree of genetic diversity. Thirdly, based on work done with other species (Hughes and Stachowicz, 2004; Ehlers et al., 2008; Reynolds et al., 2012) we might expect that high genetic diversity in *Z. japonica* would cause these beds to be resilient to environmental perturbations and may promote continued population expansion in the region (Kendrick et al., 2012).

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### References

- Abe, M., Yokota, K., Kurashima, A., Maegawa, M., 2009. Temperature characteristics in seed germination and growth of *Zostera japonica* Ascherson and Graebner from Ago Bay, Mie Prefecture, central Japan. *Fish Sci.* 75, 921–927.
- Ailstock, M.S., Shafer, D., Magoun, D., 2010. Factors affecting seed viability during processing and storage of *Potamogeton perfoliatus* and *Ruppia maritima* seeds collected from wild populations in Maryland, USA. *Restor. Ecol.* 18, 560–573.
- Alexandre, A., Cabaco, S., Santos, R., Serrao, E.A., 2006. Timing and success of reproductive stages in the seagrass *Zostera noltii*. *Aquat. Bot.* 85, 219–223.
- Baskin, C.C., Baskin, J.M., 1998. *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press, New York.
- Benech-Arnold, R.L., Sanchez, R.A. (Eds.), 2004. *Food Products Press: Haworth Reference Press*, New York.
- Bigley, R.E., 1981. The population biology of two intertidal seagrasses, *Zostera japonica* and *Ruppia maritima* at Roberts Bank, British Columbia. University of British Columbia. Thesis, 216 pp.
- Burt, W.V., McAlister, W.B., 1959. Recent studies of the hydrography of Oregon estuaries. *Res. Briefs. Fish Commission Oregon* 7, 14–27.
- den Hartog, C., 1970. *The Sea-Grasses of the World*. North-Holland Publishing Company, Amsterdam, Netherlands.
- Dooley, F.D., Wyllie-Echeverria, S., Van Volkenburgh, E., 2013. Long-term seed storage and viability of *Zostera marina*. *Aquat. Bot.* 111, 130–134.
- Ehlers, A., Worm, B., Reusch, T.B.H., 2008. Importance of genetic diversity in eelgrass *Zostera marina* for its resilience to global warming. *Mar. Ecol. Prog. Sers.* 355, 1–7.
- Ellner, S., 1986. Germination dimorphisms and parent-offspring conflict in seed germination. *J. Theor. Biol.* 123, 173–185.
- Emmett, R., Llanso, R., Newton, J., Thom, R., Hornberger, M., Morgan, C., Levings, C., Copping, A., Fishman, P., 2000. Geographic signatures of North American West Coast Estuaries. *Estuaries* 23, 765–792.
- Freund, R.J., Wilson, W.J., 1993. *Statistical Methods*. Academic Press, Inc., San Diego, California.

- Harper, J.L., 1977. Population Biology of Plants. Academic Press, London.
- Harrison, P.G., 1979. Reproductive strategies in intertidal populations of two co-occurring seagrasses (*Zostera* spp.). Can. J. Botany, 57, 2635–2638.
- Harrison, P.G., 1982a. Spatial and temporal patterns in abundance of two intertidal seagrasses, *Zostera americana* den Hartog and *Z. marina* L. Aquat. Bot. 12, 305–320.
- Harrison, P.G., 1982b. Seasonal and year-to-year variation in mixed intertidal populations of *Zostera japonica* Aschers. & Graebn. and *Ruppia maritima* L.S.L. Aquat. Bot. 14, 371–373.
- Harrison, P.G., 1991. Mechanisms of seed dormancy in an annual population of *Zostera marina* (eelgrass) from The Netherlands. Can. J. Bot. 69, 1972–1976.
- Harrison, P.G., Bigley, R.E., 1982. The recent introduction of the seagrass *Zostera japonica* Aschers. & Graebn. to the Pacific Coast of North America. Can. J. Fish Aquat. Sci. 39, 1642–1648.
- Henderson, J.S., 2014. Direct Effects and Tradeoffs Affect Vegetative Growth and Sexual Reproduction in an Invasive Seagrass Experiencing Different Disturbance Regimes. Masters Thesis. Oregon State University, Corvallis, OR, USA.
- Hootsmans, M.J.M., Vermaat, J.E., van Vierssen, W., 1987. Seedbank development, germination and early seedling survival of two seagrass species from The Netherlands: *Zostera marina* L., and *Zostera noltii* Hornem. Aquat. Bot. 28, 275–285.
- Hughes, A.R., Stachowicz, J.J., 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. P. Natl. Acad. Sci. [www.pnas.org/cgi/doi/10.1073/pnas.0402642101](http://www.pnas.org/cgi/doi/10.1073/pnas.0402642101).
- Hughes, A.R., Stachowicz, J.J., 2009. Ecological impacts of genotypic diversity in the clonal seagrass *Zostera marina*. Ecology 90, 1412–1419.
- Jarvis, J.C., Moore, K.A., 2010. The role of seedlings and seed bank viability in the recovery of Chesapeake Bay, USA, *Zostera marina* populations following a large-scale decline. Hydrobiologia 649, 55–68, <http://dx.doi.org/10.1007/s10750-010-0258-z>
- Jarvis, J.C., Moore, K.A., Kenworthy, W.J., 2014. Persistence of *Zostera marina* L. (eelgrass) seeds in the sediment seed bank. J. Exp. Mar. Biol. Ecol. 459, 126–136.
- Kaldy, J.E., 2006. Production ecology of the non-indigenous seagrass, dwarf eelgrass (*Zostera japonica* Aschers & Graebn) in a Pacific Northwest estuary, USA. Hydrobiologia 553, 210–217.
- Kaldy, J.E., Shafer, D.J., 2012. Effects of salinity on survival of the exotic seagrass *Zostera japonica* subjected to extreme high temperature stress. Bot. Mar. 56, 72–82.
- Kaldy, J.E., Shafer, D.J., Magoun, A.D., 2015. Duration of temperature exposure controls growth of *Zostera japonica*: implications for zonation and colonization. J. Expt. Mar. Biol. Ecol. 464, 68–74.
- Kendrick, G.A., Waycott, M., Carruthers, T.J.B., Cambridge, M., Hovey, R., Krauss, S.L., Lavery, P.S., Les, D.H., Lowe, R.J., Vidal, O.M.I., Ooi, J.L.S., Orth, R.J., Rivers, D.O., Ruiz-Montoya, L., Sinclair, E.A., Statton, J., van Dijk, J.K., Verduin, J.J., 2012. The central role of dispersal in the maintenance and persistence of seagrass populations. BioScience 62, 56–65.
- Koch, G.G., Atkinson, S.S., Stokes, M.E., 1986. Poisson regression. In: Kotz, S., Johnson, N.L., Read, C.B. (Eds.), Encyclopedia of Statistical Sciences. John Wiley, New York, pp. 6207–6215.
- Lee II, H., Brown, C.A. (Eds.), 2009. U.S. EPA, Office of Research and Development, Nation Health and Environmental Effects Research Laboratory, Western Ecology Division, EPA/600/R-09/140.
- Loques, F., Caye, G., Meinesz, A., 1990. Germination in the marine phanerogam *Zostera noltii* Hornemann at Golfe Juan, French Mediterranean. Aquat. Bot. 38, 249–260.
- Mach, M.E., Wyllie-Echeverria, S., Chan, K.M.A., 2014. Ecological effect of a nonnative seagrass spreading in the Northeast Pacific: a review of *Zostera japonica*. Ocean Coastal Manage. 102, 375–382.
- McMahon, K., van Dijk, K.J., Ruiz-Montoya, L., Kendrick, G.A., Krauss, S.L., Waycott, M., Verduin, J., Lowe, R., Statton, J., Brown, E., Duarte, C., 2014. The movement ecology of seagrasses. Proc. R. Soc. B 281, 20140878.
- Morita, T., Miyamatsu, A., Fujii, M., Kokubu, H., Abe, M., Kurashima, A., Maegawa, M., 2011. Germination in *Zostera japonica* is determined by cold stratification, tidal elevation and sediment type. Aquat. Bot. 95, 234–241.
- Orth, R.J., Fishman, J.R., Harwell, M.C., Marion, S.R., 2003. Seed-density effects on germination and initial seedling establishment in eelgrass *Zostera marina* in the Chesapeake Bay region. Mar. Ecol. Prog. Sers. 250, 71–79.
- Orth, R.J., Harwell, M.C., Bailey, E.M., Bartholomew, A., Jawad, J.T., Lombana, A.V., Moore, K.A., Rhode, J.M., Woods, H.E., 2000. A review of issues in seagrass seed dormancy and germination: implications for conservation and restoration. Mar. Ecol. Prog. Sers. 200, 277–288.
- Orth, R.J., Harwell, M.C., Inglis, G.J., 2006. Ecology of seagrass seeds and dispersal strategies. In: Larkum, A.W.D., Orth, R.J., Duarte, C.M. (Eds.), Seagrasses: Biology, Ecology, and Conservation. Springer, New York, pp. 111–133.
- Ott, R.L., Longnecker, M., 2001. An Introduction to Statistical Methods and Data Analysis, 5th Edition. Duxbury, pp. 904–912.
- Park, S.R., Kim, Y.K., Kim, J.H., Kang, C.K., Lee, K.S., 2011. Rapid recovery of the intertidal seagrass *Zostera japonica* following intense Manila clam (*Ruditapes philippinarum*) harvesting activity in Korea. J. Expt. Mar. Biol. Ecol. 407, 275–283.
- Procaccini, G., Olsen, J.L., Reusch, T.B.H., 2007. Contribution of genetics and genomics to seagrass biology and conservation. J. Expt. Mar. Biol. Ecol. 350, 234–259.
- Raghavan, V., 2000. Developmental Biology of Flowering Plants. Springer, New York.
- Reusch, T.B.H., Stam, W.T., Olsen, J.L., 2000. A microsatellite-based estimation of clonal diversity and population subdivision in *Zostera marina*, a marine flowering plant. Mol. Ecol. 9, 127–140.
- Reynolds, L.K., McGlathery, K.J., Waycott, M., 2012. Genetic diversity enhances restoration success by augmenting ecosystem services. PLoS One 7 (6), e38397, <http://dx.doi.org/10.1371/journal.pone.0038397>
- Ruckelshaus, M.H., 1996. Estimation of genetic neighborhood parameters from pollen and seed dispersal in the marine angiosperm, *Zostera marina*. Evolution 50, 856–864.
- Ruesink, J.L., Hong, J.S., Wisehart, L., Hacker, S.D., Dumbauld, B.R., Hession-Lewis, M., Trimble, A.C., 2010. Congener comparison of native (*Zostera marina*) and introduced (*Z. japonica*) eelgrass at multiple scales within a Pacific Northwest estuary. Bio. Invasions 12, 1773–1789.
- Schupp, E.W., 1995. Seed-seedling conflicts, habitat choice, and patterns of plant recruitment. Am. J. Bot. 82, 399–409.
- Shafer, D.J., 2007. Physiological factors affecting the distribution of the nonindigenous seagrass *Zostera japonica* along the Pacific coast of North America. In: Dissertation. University of South Alabama.
- Shafer, D.J., Wyllie-Echeverria, S., Sherman, T.D., 2008. The potential role of climate in the distribution and zonation of the introduced seagrass *Zostera japonica* in North America. Aquat. Bot. 89, 297–302.
- Shafer, D.J., Kaldy, J.E., Sherman, T.D., Marko, K.M., 2011. Effects of salinity on photosynthesis and respiration of the seagrass *Zostera japonica*: a comparison of two established populations in North America. Aquat. Bot. 95, 214–220.
- Shafer, D.J., Kaldy, J.E., 2014. Comparison of photosynthetic characteristics of the seagrass congeners *Zostera marina* L. and *Zostera japonica* Ascher. & Graeb. Aquat. Bot. 112, 91–97.
- Shafer, D.J., Kaldy, J.E., Gaeckle, J., 2014. Science and management of the introduced seagrass *Zostera japonica* in North America. Environ. Manage. 53, 147–162.
- Starrfelt, J., Kokko, H., 2010. Parent-offspring conflict and the evolution of dispersal distance. Am. Nat. 175, 38–49.
- Stokes, M.E., Davis, C.S., Koch, G.G., 2000. Categorical Data Analysis Using the SAS System, 2nd ed. SAS Institute, Inc., Cary, North Carolina.
- Trivers, R.L., 1974. Parent-offspring conflict. Am. Zool. 14, 249–264.
- Uma Shaanker, R., Ganeshiah, K.N., Bawa, K.S., 1988. Parent-offspring conflict, sibling rivalry and brood size patterns in plants. Ann. Rev. Ecol. Syst. 19, 177–205.
- Wyllie-Echeverria, S., Ackerman, J.D., 2003. The seagrasses of the Pacific Coast of North America. In: Green, E.P., Short, F.T. (Eds.), World Atlas of Seagrasses. University of California Press, Berkeley, California, pp. 199–206.
- Wyllie-Echeverria, S., Wyllie-Echeverria, V.R., Churchill, A.C., Cox, P.A., 2006. Further evidence for seed size variation in the genus *Zostera*: exploratory studies with *Zostera japonica* and *Zostera asiatica*. Aliso 22, 241–245.
- Zar, J.H., 1996. Biostatistical Analysis. Prentice Hall, Upper Saddle River, New Jersey.
- Zipperle, A.M., Coyer, J.A., Reise, K., Stam, W.T., Olsen, J.L., 2009. Evidence for persistent seed banks in dwarf eelgrass *Zostera noltii* in the German Wadden Sea. Mar. Ecol. Prog. Sers. 380, 73–80.