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Effects of salinity on the growth and morphology of the invasive, euryhaline hydroid *Cordylophora* (Phylum Cnidaria, Class Hydrozoa)

Nadine C. Folino-Rorem,[a] and Corbin J. Renken[b]

Department of Biology, Wheaton College, Wheaton, Illinois 60187, USA

Abstract. The invasive, euryhaline hydroid *Cordylophora* sp. is a colonial cnidarian present in both freshwater and brackish water habitats. Individuals contend with osmotic stress at the tissue and cellular level. It has been suggested that this hydroid's ability to expand its range of distribution by invading new habitats is due in large part to an ability to acclimate to new salinities. The purpose of this study was to assess colony growth and morphological changes at various salinities in freshwater and brackish genotypes of *Cordylophora* sp. Single genotypes from a known freshwater clade (0.5 psu; Des Plaines River) and a known brackish clade (16 psu; Napa River) were cultured and gradually transitioned to 12 different salinities ranging 0.5–22 psu, and we characterized the growth rates and hydranth morphological features at each salinity. Colony growth was optimal at 0.5 psu for the freshwater genotype and 10 psu for the brackish genotype. Changes in hydranth morphology in the freshwater genotype were primarily observed at higher salinities, while morphological changes in the brackish genotype primarily occurred at lower salinities. Our results for the brackish genotype generally concur with previous work, but this study is the first to document the response of a freshwater genotype of *Cordylophora* sp. to various salinities. Differences in growth between these two genotypes strongly support the previously proposed existence of multiple cryptic species. Furthermore, because this hydroid is quite prevalent in freshwater and brackish systems as a fouling organism, understanding the effects of various salinities on the successful establishment of *Cordylophora* sp. is an important contribution to the understanding of the ecophysiology and management of this invasive hydroid.

Additional key words: *Cordylophora*, genotypes, hydroid, osmoregulation

Salinization is a topic of growing concern for aquatic ecologists because increasing salt concentrations are affecting the biodiversity and community structure of aquatic ecosystems worldwide (Kaushal et al. 2005; Kalaus & Ojaveer 2014; Kefford et al. 2016). A significant consequence of salinization for organisms in affected habitats is the need to deal with osmotic stress. Organisms in freshwater environments have a lower tolerance for salinity fluctuations compared with those in brackish systems (Williams 2001).

There have been few studies considering osmotic stress in sessile organisms such as cnidarians that lack organ systems and primarily deal with osmotic stress at the cellular and tissue levels (Kinne 1958; Steinbach 1963; Prusch et al. 1976; Amado et al. 2011). Moreover, most studies on cnidarian ecophysiology address salinity stress in marine and brackish species, given the greater prevalence of cnidarians in these habitats compared with freshwater habitats (Jankowski et al. 2008; Folino-Rorem 2015). Relatively few studies have assessed the osmotic stress of cnidarians present in freshwater habitats, which may be greater compared with brackish or marine systems (Williams 2001). Most freshwater studies have focused on the solitary hydroid *Hydra* (Lilly 1955; Steinbach 1963; Benos & Prusch 1973) and the medusa stage of the hydrozoan *Craspedacusta* (Fleming & Hazelwood 1967; Hazelwood et al. 1970). However, euryhaline cnidarians are especially interesting subjects for research, as they often occur in habitats such as estuaries that have fluctuating salinities. Unlike many other animals present in these environments, they lack organ systems to perform osmoregulation. One such

[a]Author for correspondence.
E-mail: nadine.rorem@wheaton.edu
[b]Present address: Marine Biological Laboratory, Massachusetts 02543, USA

cnidarian is the invasive, euryhaline hydroid *Cordylophora* sp. that is found in both freshwater and brackish habitats ranging 0–22 psu (Arndt 1984; Jankowski et al. 2008; Folino-Rorem et al. 2009). To our knowledge, no one has addressed osmotic stress as it relates to growth and morphology in freshwater populations of *Cordylophora*.

Cordylophora sp. is hypothesized to have originated from the Ponto-Caspian region (the Black, Caspian, and Sea of Azov and surrounding aquatic habitats) with an expansion in range due to transport via ship ballast or ship fouling (see review Folino-Rorem 2015). The ability to tolerate and become established within a range of salinities is an important feature of Ponto-Caspian invaders that contend with new salinities in novel habitats (Berezina & Panov 2004; Paavola et al. 2005; Grabowski et al. 2009; Dobrzycka-Krahel & Graca 2014). *Cordylophora* is present on all continents except Antarctica, and its extensive global occurrence is due presumably, in part, to the robust salinity tolerance and acclimating abilities of the hydroids (bij de Vaate et al. 2002; Folino-Rorem 2015).

Colonies of *Cordylophora* sp. consist of individual feeding polyps called hydranths that grow from branched stalks known as hydrocauli (Fraser 1944) (Fig. 1). Each hydrocaulus is connected via the hydrorhizae that anchor the colonies to the substrate (Folino-Rorem 2015). Colonies are dioecious and produce either male or female reproductive structures, called gonophores. A medusa stage is lacking, and fertilized eggs develop into free-swimming planula larvae that settle to form sessile, primary polyps. Colonies reproduce asexually by budding and can be quite prolific depending on the time of year (Muskó et al. 2008; Folino-Rorem 2015). Temperature and salinity have been shown to significantly influence growth rates (Kinne 1956), and a wide range of salinities have been reported as optimal for colony growth (Kinne 1956; Blezard 1999; Chester et al. 2000).

Hydranth morphology also varies with salinity. Kinne (1958) observed that hydranths of a brackish genotype became shorter and wider or longer and narrower when cultured in freshwater or salt water, respectively. In addition, the tentacles of hydranths were longest at brackish salinities and became shorter when reared in freshwater or salt water (Kinne 1958). The number of tentacles per hydranth typically ranges 14–16, although some hydranths have been observed with as many as 27; this too varies with salinity (Kinne 1958; Schuchert 2004). Historically, the number, type, and arrangement of tentacles on the hydranth have been the primary diagnostic features used in taxonomy.

The noteworthy morphological plasticity of *Cordylophora* in response to salinity has contributed to inconsistencies in taxonomic nomenclature and created uncertainty over the systematic status of the genus (Schuchert 2004; Folino-Rorem 2015). The taxonomy of this hydroid at the family and genus level is in a tentative state. Assigning the family

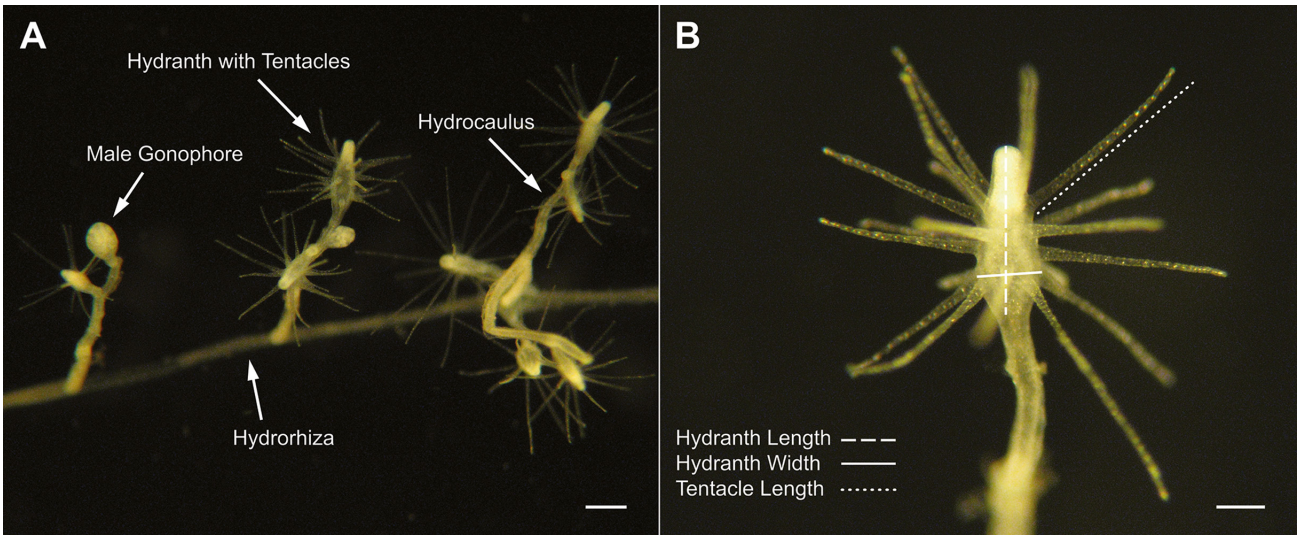

Fig. 1. The invasive, euryhaline colonial hydroid *Cordylophora*. **A.** The anatomy of a colony. Colonies of *Cordylophora* grow by producing horizontal hydrorhizae over a substrate. From these extend vertical hydrocauli, each of which may bear few or many feeding hydranths and reproductive gonophores. **B.** A close-up detail of a hydranth highlighting the morphological features assessed in this study. Scale: A=400 μm; B=200 μm.

name of Oceaniidae or Clavidae or the more historically accurate name of Cordylophoridae at this point in time is suggested until more taxonomic work is conducted to clarify the family's status (D. Calder and P. Schuchert, unpubl. data; Folino-Rorem 2015). Recent molecular research assessing a global range of populations from freshwater and brackish habitats suggests that multiple clades of *Cordylophora* exist and also perhaps multiple cryptic species of *Cordylophora* (Folino-Rorem et al. 2009). Although the current practice in the literature is to refer to both freshwater and brackish populations as *C. caspia* (Pallas 1771), we will only use the genus name throughout this paper. It is evident that the taxonomy of the genus *Cordylophora* is in need of a thorough reexamination.

It is ecophysiological plasticity that enables *Cordylophora* to survive broad changes in salinity, and this is an important factor in assessing the ability of this hydroid to invade nonnative waters (Leppäkoski et al. 2002; Ma & Purcell 2005; Folino-Rorem 2015). Therefore, it is advantageous to understand the osmoregulative capabilities of different populations of *Cordylophora* and how these relate to observed distribution patterns (Folino-Rorem et al. 2009). The purpose of this study was to assess osmotic stress and physiological flexibility in fresh and brackish water clades of *Cordylophora* in various salinities ranging 0–22 psu. The effects of these salinities were quantified by examining colony growth and variation in hydranth morphology. This study is unique in that it is the first to consider osmotic stress not only in a brackish genotype but also in a freshwater genotype.

Methods

Hydroid collecting

We collected two riverine populations of *Cordylophora* and established stock colonies in the laboratory: a clade 1A freshwater genotype from the Des Plaines River (0.5 psu) in Joliet, IL, USA (41°31′30.70″N, 088°05′11.13″W); and a clade 2B brackish genotype from the Napa River (16 psu) in Napa, CA, USA (38°11′49.87″N, 122°18′57.59″W). Each stock colony was grown from a single polyp as described by Fulton (1962), resulting in two genetically homogenous stock colonies that were maintained in the lab for 1–2 years before the present study. Samples of DNA from both stock colonies were sequenced as described in Folino-Rorem et al. (2009) to determine their genetic identity (freshwater genotype, clade 1A; brackish genotype, clade 2B), and both were reared and maintained at the salinity of their original collection sites.

Salinity effects on growth

Culturing colonies. To establish replicate colonies for experimentation, pieces with two hydranths each were obtained from the stock cultures and tied on microscope slides (25×75 mm) (Fulton 1962). Twenty (n=20) replicate slides for each control and experimental salinity treatment group were placed in plastic slide trays (14.8×8.8 cm; five slides per slide tray) with the top and bottom cut out allowing for water flow across the slides. Each slide was placed in the tray with the colony face down to prevent debris build-up. Thirty-six trays with five colonies each (a total of 180 slides) were established at 0.5 psu with the freshwater genotype. Another 48 trays with five colonies each (a total of 240 slides) were established at 16 psu with the brackish genotype. All colonies were reared in water mixed from a standard solution of CCS5 (0.05 mol L^{-1} NaCl, 0.001 mol L^{-1} KCl, 0.005 mol L^{-1} $CaCl_2$, 0.005 mol L^{-1} $MgCl_2$, 0.001 mol L^{-1} $NaHCO_3$) as described by Fulton (1962). We dissolved CCS5 ions in double deionized (DDI) water purified from a Purelab® Ultra (Elga High Wycombe, UK) water purifier, yielding a salinity of 4.1 psu. To obtain lower salinities, this solution was diluted with DDI water, while higher salinities were obtained by adding Instant Ocean® sea salt (Spectrum Brands, Blacksburg, VA, USA).

Acclimation. Colonies of both genotypes were acclimated from their original salinity to new experimental salinities in 2 psu increments over the course of 2 weeks. Pilot studies (Folino-Rorem, unpubl. data) indicated that abrupt changes in salinity stymie colony recovery and growth; therefore, colonies were transitioned across salinities incrementally in a stepwise manner with a 2-d period of acclimation between each change. The acclimation process was staggered so that each treatment group reached its target salinity on the same day. Twenty colonies from each genotype remained in their original salinity to serve as a control (0.5 psu for the freshwater and 16 psu for the brackish). The remaining freshwater colonies were transitioned to nine different experimental salinities over the range of 0.5, 2, 4, 6, 8, 10, 12, 14, and 16 psu, while the brackish colonies were transitioned to 12 different experimental salinities ranging 0.5–22 psu (the smaller range for the freshwater colonies was based on preliminary results indicating minimal growth for this genotype above 16 psu).

Two replicate 2.5-L aquaria were used to house each treatment group. The four slide trays of each group were split between the two replicate tanks to control for any tank effect. During the 2-week acclimation period, colonies were fed to saturation with *Artemia* nauplii for 15–20 min, five times per week. Colonies were placed in separate containers for feeding, and *Artemia* nauplii were presented already suspended in water of the corresponding salinity for each treatment group.

All colonies were housed in a temperature-controlled room (~22°C) under constant water flow achieved by equipping each aquarium with an AquaClear™110 side filter (Mansfield, MA, USA). Each slide tray was rotated in position within the tank daily to control for potential effects due to water flow differences. Salinity was monitored daily and adjusted accordingly.

Measuring growth. Once the treatment groups reached target salinity, the size of each replicate colony was standardized by trimming to five hydranths. Next, each treatment group was kept at its target salinity and monitored for growth for 2 or 3 weeks. Due to time restraints, the freshwater colonies were monitored for 15 d, while the brackish colonies were monitored for 21 d. The number of buds, hydranths, and gonophores in each replicate colony were counted three times per week using a dissecting microscope. Buds were defined as newly forming hydranths or gonophores too immature to distinguish, as well as small identifiable hydranths before developing mature tentacles. Buds were counted separately as they represented new colony growth but were either too immature to contribute to reproduction or too immature to capture prey and support the energy needs of the colony for continued growth. During this period, each colony was fed with *Artemia* nauplii three times per week as described.

Salinity effects on morphology

For both genotypes, five salinity treatment groups of 12 replicate colonies each were established and cultured as previously described and held in slide trays with six slides to a tray. Each treatment group was housed in a 1.5-L plastic tub (two trays per tub) with aerated water. Each treatment group was acclimated in 2 psu increments from its original salinity to a target salinity of 0.5, 4, 8, 12, or 16 psu (this experimental range of salinities differed from those of the growth experiments because it was conducted 1 year prior). Once colonies reached their respective treatment salinity, they were maintained there for 2 weeks before morphological measurements were recorded. Colonies were fed three times per week postacclimation.

All morphological measurements were made under a dissecting microscope equipped with an ocular micrometer. Whenever possible, measurements were taken for four hydranths per colony for a total of 48 replicates in each treatment group. In one case (the control group of 0.5 psu in the freshwater genotype), only two or three hydranths per colony were available for measurements due to insufficient growth before measuring day (these colonies needed no acclimation period and thus had a shorter time to grow). Measuring protocol was standardized by measuring four of the youngest hydranths near the growing edge of the colony, whenever possible, so as to measure hydranths reared entirely at the target salinity. This helped ensure that measurements accurately reflected the morphological response to each salinity. Hydranth length was measured as the distance between the end of the hydrocaulus and the tip of the hydranth, and width as the widest portion of a given hydranth (Fig. 1B). The tentacle number was counted for each selected hydranth. The lengths of the four longest tentacles on each hydranth were measured and averaged to give one value per hydranth.

Data analyses

Assessing growth. To obtain mean growth rates over time for comparison between the freshwater and brackish genotypes, we plotted the total number of polyps (hydranths, buds, and gonophores combined) and the number of hydranths and buds combined per colony over time for each replicate slide. We did not calculate growth rates for gonophores because production was highly variable and did not demonstrate a linear relationship over time. For both genotypes, we used linear regression to estimate 20 replicate growth rates for each salinity group. Replicates lacking a statistically significant relationship between polyp number and time ($p>0.05$) were considered outliers and not included in further analyses. The mean slopes for each treatment group were plotted to determine the salinity of optimal growth for both genotypes.

Assessing hydranth morphology. Hydranth length, hydranth width, tentacle length, and tentacle number were recorded for four hydranths per colony. For both genotypes, we measured 12 colonies per salinity treatment for a total of 48 replicates in each treatment group. To check for outliers in the control groups, the values in these groups were converted to

Z-scores. Replicates with a score greater than |3| were considered outliers and were excluded in further analyses. Using this method, one outlier was found in the freshwater hydranth width control group, and the features of this hydranth were not considered in hydranth length, width, and tentacle length, or number. One outlier was found in the brackish control group for hydranth length and was similarly excluded from further analyses.

To check for outliers in the experimental groups, comparisons were made with and without the replicate in question. If the replicate changed the significance of the result, it was excluded from all analyses. No replicates in the experimental groups were deemed outliers and excluded using this method.

When it came time to quantify the hydranths of the freshwater control group, many of the colonies lacked enough hydranths to measure four replicates each. This was due to an oversight in experimental design that only gave these colonies 2 weeks to grow between being established on slides and measuring day. While these colonies were maintained and monitored for health throughout the duration of the experiment, they were not re-measured due to time constraints. Therefore, the number of replicates in the freshwater control group was approximately half of the other data sets. We chose to use a robust nonparametric test due to the smaller sample size in the freshwater control group and the occasional departures from a normal distribution observed in some of our data sets. Wilcoxon rank-sum tests were conducted to compare the responses of each experimental group to the control using the statistical program JMP (version 12.1), which provided the Z-test statistic for a normal approximation test and the corresponding p-value.

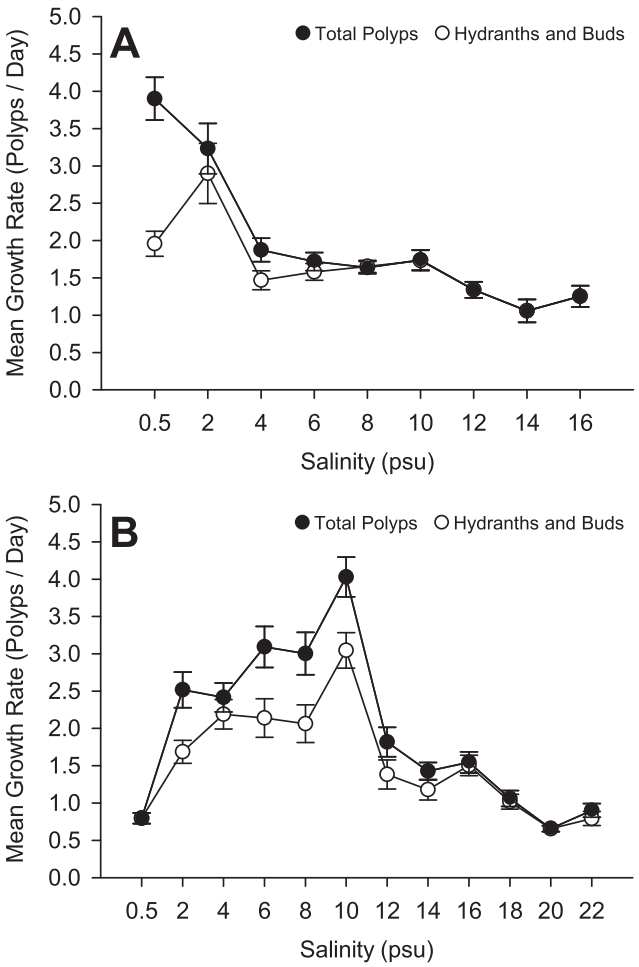

Fig. 2. Colony growth rates vary with changes in salinity. Mean growth rates are presented, including and excluding reproductive gonophore polyp counts ("Total Polyps" and "Hydranths and Buds," respectively). **A.** Freshwater genotype (from Des Plaines River). The mean growth rates in the Joliet colonies decreased with increasing salinity. Control is 0.5 psu. Growth was measured over 15 d. **B.** Brackish genotype (Napa River). The mean growth rate in the Napa colonies peaked at 10 psu, decreasing at salinities above and below. Control is 16 psu. Growth was measured over 21 d. Note difference in salinity ranges between A and B. All means were calculated from 15 to 20 replicates. Error bars indicate ±SE.

Results

Colony growth and reproduction

Both genotypes of *Cordylophora* exhibited changes in colony growth rate relative to salinity (Fig. 2). The total polyp growth rate of the freshwater colonies from Joliet, IL, was optimal at the control salinity of 0.5 psu (mean growth rate=3.90±0.287 polyps d^{-1}, mean±SE) (Fig. 2A). The production of hydranths and buds alone was lower, with a mean rate of 1.96±0.168 polyps d^{-1}, revealing a relatively high rate of gonophore production at the control salinity (Fig. 2A). There was a steady decline in the mean growth rate of total polyps and of hydranths and buds combined as the treatment salinity increased (Fig. 2A). At the control salinity of 0.5 psu, the mean number of gonophores per colony was greatest after 10 d (27.8±2.33 gonophores) and 13 d (23.9±1.93 gonophores). The presence of gonophores decreased as the salinity increased, with almost no gonophore production at 12, 14, and 16 psu (Fig. 2A).

The optimal growth rate in terms of total number of polyps in the brackish colonies from Napa, CA, occurred at 10 psu, with a mean rate of 4.03±0.268

polyps d^{-1} (Fig. 2B). This was greater than that at the control salinity of 16 psu (mean rate=1.54±0.141 polyps d^{-1}). In addition, there was a steady decline in the mean growth rates at salinities higher and lower than the optimal salinity of 10 psu (Fig. 2B). In contrast to the freshwater genotype, brackish colonies produced gonophores at all salinities from 0.5 to 22 psu, with the highest production at 2, 4, 6, 8, 10, and 12 psu. The greatest prevalence of gonophores occurred at 10 psu after 3 weeks of growth, with a mean of 20.7±0.966 gonophores (n=20) per colony. The higher prevalence of gonophores at these salinities is reflected in the differences between the growth rates calculated using the total number of polyps and using hydranths and buds alone (Fig. 2B).

Hydranth morphology

Both genotypes of *Cordylophora* demonstrated hydranth morphological plasticity in response to salinity. The mean hydranth lengths in both genotypes became significantly longer when acclimated away from their controls (Fig. 3). In the freshwater genotype, the mean hydranth lengths were significantly longer than the control (0.5 psu) (647±51.3 μm, mean±SE) in treatments at 8 psu (Z=4.84, p<0.0001), 12 psu (Z=3.78, p=0.0002), and 16 psu (Z=4.02, p<0.0001) (Fig. 3A). The mean hydranth length at 4 psu was not significantly longer than the control (Z=1.88, p=0.06). The mean hydranth length was greatest at 8 psu (1030±37.6 μm). The trend in hydranth length was directly related to salinity for this genotype (Fig. 3A). Conversely, in several low-salinity treatments, the mean hydranth lengths in the brackish genotype were significantly longer than mean hydranth length in the control (16 psu) (702±24.4 μm): at 0.5 psu (Z=−8.40, p<0.0001); at 4 psu (Z=−7.73, p<0.0001); at 8 psu (Z=−7.29, p<0.0001); and at 12 psu (Z=−6.87, p<0.0001) (Fig. 3B). The mean hydranth length was greatest at 0.5 psu (1810±52.2 μm). The trend in hydranth length was inversely related to salinity for this genotype (Fig. 3B).

The mean hydranth widths were shorter with increasing salinity in the freshwater genotype, but lacked a discernable pattern in the brackish genotype (Fig. 3). Mean hydranth widths for the freshwater genotype were significantly narrower than the control (0.5 psu) (322±19.3 μm) at 8 psu (Z=−2.11, p=0.03), 12 psu (Z=−3.48, p=0.0005), and 16 psu (Z=−5.15, p<0.0001) (Fig. 3A). The mean width at 4 psu was not significantly smaller than the control

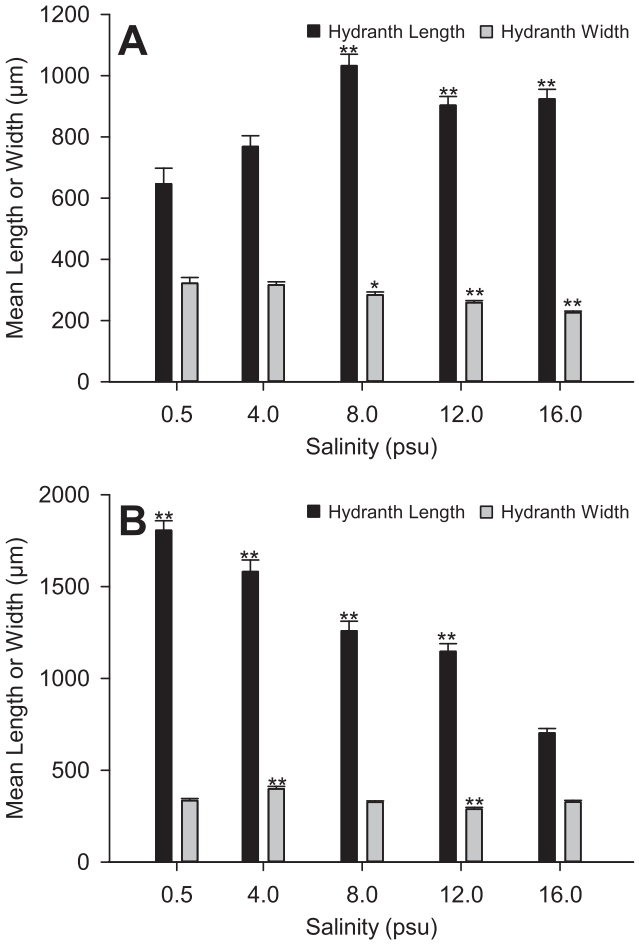

Fig. 3. Mean hydranth length and width vary from the control 2 weeks after acclimation to a new treatment salinity. **A.** Freshwater genotype. The mean hydranth length in the Joliet colonies increased with increasing salinities, while the mean width decreased. Control is 0.5 psu. **B.** Brackish genotype. The mean hydranth length in the Napa colonies increased with decreasing salinities, while the mean width lacked a discernable pattern. Control is 16 psu. Note difference in scale between A and B. Means at 0.5 psu in A were calculated from n=24–25 replicates. All other means were calculated from n=43–48 replicates. Error bars indicate SE. *p<0.05, **p<0.01.

(Fig. 3A). In the brackish genotype, the mean hydranth widths were significantly different than the control (16 psu; 326±9.33 μm) at 4 and 12 psu (Fig. 3B). The hydranths at 4 psu were significantly wider than the control (Z=−3.90, p<0.0001), while those at 12 psu were narrower (Z=2.67, p=0.008) (Fig. 3B). The differences in mean hydranth width lacked a discernable pattern in this genotype, unlike the inverse relationship between width and salinity observed in the freshwater genotype (Fig. 3).

Mean tentacle length was shorter and then longer than the control treatment with increasing salinity in

the freshwater genotype, whereas tentacle length was longer with decreasing salinity in the brackish genotype (Fig. 4). The mean tentacle lengths in the freshwater genotype were significantly longer than the control (0.5 psu) (718±65.5 μm) at 8 psu ($Z=6.75$, $p<0.0001$), 12 psu ($Z=6.09$, $p<0.0001$), and 16 psu ($Z=6.69$, $p<0.0001$) (Fig. 4A). At 4 psu, the mean tentacle length was significantly shorter ($Z=-2.70$, $p=0.007$). The mean tentacle length was greatest at 16 psu (1650±47.1 μm). With the exception of the 4 psu treatment, the freshwater genotype exhibited an approximately direct relationship between salinity and tentacle length (Fig. 4A). In the brackish genotype, the mean tentacle lengths were significantly longer than in the control (16 psu) (1150±58.9 μm) at 0.5 psu ($Z=-5.80$, $p<0.0001$), 4 psu ($Z=-6.73$, $p<0.0001$), 8 psu ($Z=-5.87$, $p<0.0001$), and 12 psu ($Z=-5.18$, $p<0.0001$) (Fig. 4B). The mean tentacle length was greatest at 4 psu (1980±70.2 μm). The brackish genotype exhibited an approximately inverse relationship between salinity and tentacle length (Fig. 4B).

The mean number of tentacles per hydranth was greater with increasing salinity in the freshwater genotype, but lacked a discernable pattern relative to salinity in the brackish genotype (Fig. 5). Mean tentacle numbers in the freshwater genotype were significantly greater than in the control (0.5 psu) (14.2±0.749) at 4 psu ($Z=4.37$, $p<0.0001$), 8 psu ($Z=5.37$, $p<0.0001$), 12 psu ($Z=5.64$, $p<0.0001$), and 16 psu ($Z=4.05$, $p<0.0001$) (Fig. 5A). The mean number of tentacles per hydranth was greatest at 12 psu (20.4±0.374). Overall, the freshwater genotype displayed an approximately direct relationship between salinity and mean tentacle number (Fig. 5A). In the brackish genotype, the mean tentacle numbers were significantly greater than in the control (16 psu) (21.3± 0.450) at 4 psu ($Z=-4.88$, $p<0.0001$) and at 8 psu ($Z=-3.73$, $p=0.0002$) (Fig. 5B). The mean number of tentacles per hydranth was the same, however, at 0.5 psu and at 12 psu. The number of tentacles per hydranth was greatest at 4 psu (24.5±0.373). While the mean tentacle number in the brackish genotype increased at some decreased salinities, it lacked a strong discernable relationship to salinity (Fig. 5B).

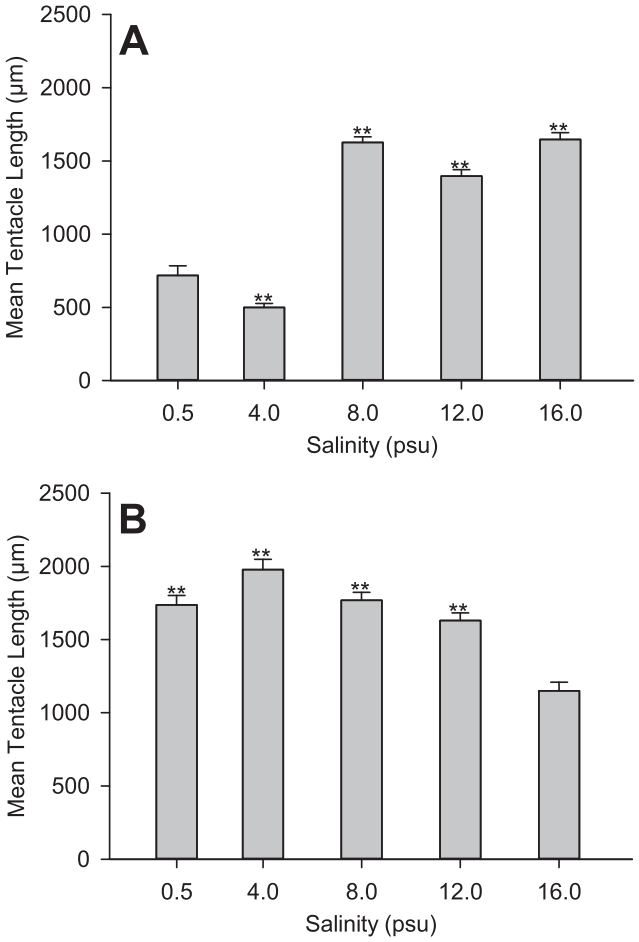

Fig. 4. Mean tentacle length varies from the control 2 weeks after acclimation to a new treatment salinity. **A.** Freshwater genotype. The mean tentacle length in the Joliet colonies decreased and then increased with increasing salinities. Control is 0.5 psu. **B.** Brackish genotype. The mean tentacle length in the Napa colonies increased with decreasing salinities. Control is 16 psu. Mean at 0.5 psu in A was calculated from n=25 replicate hydranths. All other means were calculated from n=44–48 replicate hydranths. Each replicate hydranth value is an average length of the four longest tentacles on that hydranth. Error bars indicate SE. **p<0.01.

Discussion

Colony growth and reproduction

The present study is the first to compare the effects of salinity on growth between freshwater and brackish genotypes of a hydrozoan. Salinity effects in cnidarians have often been evaluated by assessing asexual colony growth, quantified as changes in polyp number (Stebbing 1981; Ma & Purcell 2005; Sokołowski et al. 2016). Previous studies assessing asexual growth rates of *Cordylophora* at different salinities have only used brackish populations (Kinne 1956, 1964, 1971; Fulton 1962; Meek et al. 2012). Kinne (1956, 1964, 1971) conducted extensive laboratory experiments with brackish colonies but never assessed the capabilities of freshwater colonies (O. Kinne, unpubl. data).

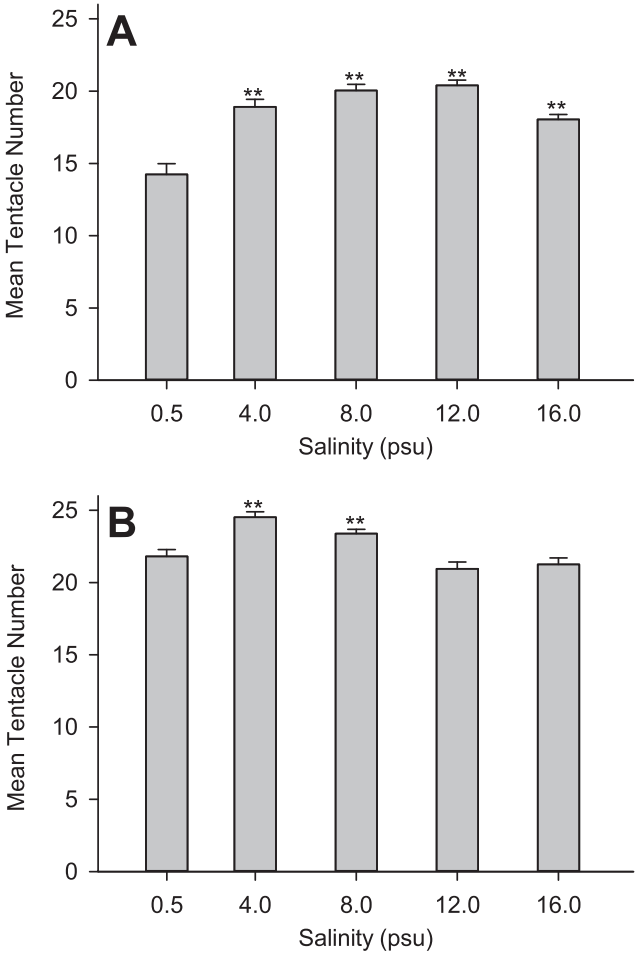

Fig. 5. Mean tentacle number varies from the control 2 weeks after acclimation to a new treatment salinity. **A.** Freshwater genotype. The mean tentacle number in the Joliet colonies increased with increasing salinities. Control is 0.5 psu. **B.** Brackish genotype. The mean tentacle number in the Napa colonies increased at some decreased salinities, but lacked a discernable pattern. Control is 16 psu. Mean at 0.5 psu in A was calculated from n=25 replicates. All other means were calculated from n=43–48 replicates. Error bars indicate SE. **p<0.01.

Differences in the observed optimal growth rate range between the freshwater genotype from Joliet, IL (0.5–6 psu) (Fig. 2A), and the brackish genotype from Napa, CA (2–10 psu) (Fig. 2B), in this study can, in part, be explained by considering osmoregulation in cnidarians. The broader salinity range of growth for the brackish genotype suggests a more physiologically plastic or better adapted genotype for handling the fluctuations in salinity that characterize estuarine habitats (Khlebovich 1990; Conde et al. 2013; Obolewski et al. 2015). Freshwater populations potentially expend more energy to osmoregulate compared with brackish populations, resulting in reduced growth at sub- and supra-optimal salinities (Kinne 1958). Growth rates of the brackish colonies in this study were optimal at 10 psu (Fig. 2B), generally concurring with previous results observed by Kinne (1956, 1958). Wintzer et al. (2011) also assessed growth rates in *Cordylophora* relative to salinity (6–11 psu) and temperature using polyps from a brackish habitat of 6.5 psu (Suisun Marsh in the San Francisco Estuary). They observed changes in growth rate based on temperature, but not salinity. Perhaps, colonies in that study regularly experience salinity changes within the range of 6–11 psu due to tides and are physiologically plastic in that range. Our results strongly suggest a correlation between salinity and growth rate in both our freshwater (0.5 psu) and brackish (10 psu) genotypes. This correlation is also supported by previous studies by Kinne (1956) and Fulton (1962). Furthermore, it would be advantageous to monitor tidal and seasonal salinity fluctuations in the Napa River and conduct experiments exposing colonies to salinity changes comparable with those in nature to obtain better indications of how this brackish genotype is able to tolerate salinity variation.

The global presence of multiple distinct cryptic clades within the genus of *Cordylophora* (Folino-Rorem et al. 2009) is likely responsible for the wide range of growth rates in the literature. Folino-Rorem et al. (2009) present four clades (1A, 1B, 2A, 2B), with 1A being exclusively freshwater, 2A and 2B exclusively brackish, and 1B consisting of freshwater and brackish populations. The present study begins to address salinity effects by reporting the growth optima of verified genotypes from two (1A and 2B) of the four known clades in the genus. At 22±1°C, we observed optimal growth at a salinity of 0.5–2 psu for the freshwater 1A clade genotype from the Des Plaines River (Fig. 2A) and at 10 psu for the brackish 2B clade genotype from the Napa River (Fig. 2B).

Reproduction in hydroids is primarily influenced by temperature, nutrition, and salinity. Salinity ranges for asexual reproduction are usually broader compared with those for sexual reproduction (Fulton 1962; Kinne & Paffenhöfer 1966; Ma & Purcell 2005). We also observed differences in gonophore production between the freshwater and brackish genotypes: the brackish colonies exhibited gonophore production across a broader salinity range (2–14 psu) than the freshwater colonies (0.5–6 psu). This broader range for potential sexual reproduction may confer an invasive advantage on the brackish genotype. Gonophore production is likely linked to

salinity due to the energy demands of osmotic stress responses, and this may differ between clades. Our brackish genotype exhibited a range of optimal gonophore production (6–10 psu), with the greatest production at 10 psu (the salinity of optimal asexual growth). By contrast, Kinne (1956) observed the greatest gonophore production at a suboptimal salinity of 5 psu in a brackish genotype (clade unknown) that exhibited optimal hydranth production at 15–17 psu. Future work investigating similarities and differences between salinity effects on gonophore production in freshwater and brackish genotypes would have merit.

Morphology

The hydranths of the freshwater genotype were short and wide at low salinities and became progressively taller and narrow in more brackish water (Fig. 3A). These changes were as we had anticipated based on previous studies by Kinne (1958). In a brackish genotype of *Cordylophora*, Kinne (1958) observed short and stout hydranths in freshwater, tall and narrow hydranths at 16.7 psu, and shorter but even narrower hydranths at 30 psu, and he determined that the hydranth surface-area-to-volume ratio increased linearly with salinity. Kinne (1958) concluded that *Cordylophora* responds to salinity stresses by adjusting the surface-to-volume ratio of its cells, the shapes of which determine the overall shape of the hydranth.

The hydranth morphology changes we observed in the brackish genotype, however, were quite unexpected. Rather than becoming progressively shorter at lower salinities, these hydranths instead became significantly taller (Fig. 3B) compared with hydranths in the freshwater genotype (Fig. 3A). These results are puzzling because it would seem that producing taller hydranths (with a greater surface-to-volume ratio) in hypoosmotic environments would expose the hydroid to more of the external medium and exacerbate the problem of water diffusing into cells. It should be noted that our study did not investigate changes in the cellular architecture of the hydranths, and thus, we cannot be certain that the epithelial ectoderm cells changed shape to produce this new hydranth shape. It is possible that these tall hydranths did produce epithelial cells columnar in shape, but at a much greater number. In addition, the tentacles of the brackish genotype were generally longer at salinities lower than the control of 16 psu, while tentacles of freshwater hydranths were longer at 8, 12, and 16 psu, salinities higher than the control of 0.5 psu (Fig. 4).

The average tentacle lengths for both genotypes followed the same pattern as hydranth length: tentacles of the freshwater genotype became longer with increasing salinities (Fig. 4A), while tentacles of the brackish genotype became longer with decreasing salinities (Fig. 4B). Kinne (1958) demonstrated that tentacle length follows the same principle of cellular surface-to-volume ratio found to be at work in hydranth shape: longer tentacles have flatter, more squamous-shaped epithelial cells. In addition, while not a perfectly clear pattern in the brackish genotype colonies, the same contrasting trend between both genotypes is present in tentacle number as well (Fig. 5). It would seem that the brackish genotype unexpectedly increases its contact with the environment at low salinities, despite that its optimal salinity for growth (and presumably its physiological optimum) is at 10 psu (Fig. 2B). This response could indicate that the salinity of optimal growth is not the best indicator of a physiological optimum. Additionally, variation in tentacle number and length could influence prey capture efficiency and the types of prey caught, which could also influence overall growth rates (Kinne & Paffenhöfer 1965). Clearly, more work is necessary to understand the physiological underpinnings of morphology in *Cordylophora*. These results may be better understood by considering aspects of osmoregulation.

Osmoregulation

Cells experiencing osmotic stressors are threatened by cell lysis or shrinkage, and invertebrate and vertebrate cells alike respond by a process of volume regulation (Gilles 1987; Hoffmann et al. 2009). In hypoosmotic solutions, cnidarian cells that begin to swell reduce cell volume via a mechanism called regulatory volume decrease (RVD). Conversely, cnidarian cells in hyperosmotic solutions that shrivel regain water through regulatory volume increase (RVI) (Amado et al. 2011; Morabito et al. 2013). Most brackish and marine cnidarians are osmoconformers and deal with osmotic stress by adjusting the osmolarity of their intracellular cytosol and enteron fluids to match that of the external medium (Benson-Rodenbough & Ellington 1982). The most common mechanism for cell volume regulation is adjusting the intracellular concentration of the free amino acid pool present in the cytosol of invertebrate cells (Lange 1972; Gilles 1979; Pierce 1982). This ability has been demonstrated in the polyps of the scyphozoans *Aurelia aurita*, *Chrysaora quinquecirrha*, and *Cyanea capillata* (Webb et al. 1972); in the euryhaline anemone *Bunodosoma cavernata*

(Kasschau et al. 1984); and in the anemone *Metridium senile* (Deaton & Hoffmann 1988).

In conjunction with osmoregulation involving the cells and tissues, freshwater and marine cnidarians use the enteron to regulate fluid fluxes (Benson-Rodenbough & Ellington 1982; Amado et al. 2011; Morabito et al. 2013; del Valle et al. 2015). In contrast to brackish and marine cnidarians, individuals of the freshwater hydroid *Hydra* live in habitats that are especially hypoosmotic relative to the cells and tissues of the hydroids, and deal with ion fluxes and osmotic stress (Lilly 1955; Steinbach 1963). Individuals of *Hydra* perform osmoregulation by using the enteron cavity to collect and expel fluids hypoosmotic to the cells and tissues of the hydroid (Benos & Prusch 1972). No detailed studies have yet addressed how individuals of *Cordylophora* contend with osmotic stress. It is conceivable that freshwater hydranths of *Cordylophora* may also expel water from their enteron in a similar fashion as observed in *Hydra* (Benos et al. 1977) and other cnidarians (Benson-Rodenbough & Ellington 1982; del Valle et al. 2015). It is conceivable that colonies of *Cordylophora*, with their ability to thrive in both freshwater and brackish environments, may employ a regulatory strategy similar to that found in *Hydra* in freshwater habitats and a conformation strategy in brackish water similar to that found in euryhaline cnidarians such as the anemone *Bunodosoma cavernata* (Kasschau et al. 1984). A more comprehensive approach employing the methods of Benos & Prusch (1972), Benos et al. (1977), Benson-Rodenbough & Ellington (1982), and Kasschau et al. (1984) will be needed to address the physiological mechanisms underlying osmotic, ionic, and volume regulation in *Cordylophora*. Such a hybrid model of osmotic stress response in *Cordylophora*, if it is indeed the case, could help further explain our results.

Because the enteron functions in both osmoregulation and digestion in *Hydra*, partially digested food and valuable nutrients may be expelled during hydranth contractions, which would negatively impact growth. This phenomenon could also play a role in food assimilation efficiency and growth in *Cordylophora*. Furthermore, digestion times in brackish populations of *Cordylophora* are shorter compared to colonies exposed to freshwater and salt water (30 psu) (Kinne & Paffenhöfer 1965). Arndt (1984) also observed differences in assimilation efficiency relative to salinity in brackish colonies of *Cordylophora*. Perhaps, differences in food assimilation efficiency exist relative to salinity for freshwater genotypes and thereby impact the overall growth rate. Trade-offs likely exist between the energetics needed for osmoregulation, food digestion, and polyp production, which is a topic worthy of further study.

Taxonomy

The genotypes used in this study come from two distinct lineages: the Joliet population falls within the freshwater clade 1A, while the Napa population is from the brackish clade 2B (Folino-Rorem et al. 2009). Our data show that the freshwater and brackish genotypes from clades 1A and 2B, respectively, have markedly different salinity tolerances (Fig. 2), and that both genotypes exhibit distinct hydranth morphologies at each salinity level investigated (Figs. 3–5). Based on the previous molecular analyses and our current data highlighting these phenotypic differences, it is highly plausible that clades 1A and 2B are distinct cryptic species with plastic morphologies and unique salinity tolerances. We recommend investigating the characteristics of the other two clades (1B and 2A) and a thorough taxonomic examination of the genus *Cordylophora*. If multiple species with distinct ecophysiological capabilities exist, as our data suggest, characterizing them will be advantageous for developing a framework to investigate and predict the ecological impacts of these hydroids in new aquatic systems (Carlton 2009; Ricciardi 2015). Collecting samples of *Cordylophora* from habitats with fluctuating salinities and conducting molecular analyses in conjunction with morphological and physiological observations would enhance the investigation of evolutionary change in this genus. Additionally, addressing taxonomic issues of invasive species will provide insights into the ecological impacts of cryptic species (Ricciardi 2015; Govindarajan & Carman 2016; Ordóñez et al. 2016).

Ecological implications

The ecological impacts of *Cordylophora* are potentially extensive since the organism inhabits freshwater and brackish habitats of various types (Conde et al. 2013; Obolewski et al. 2015). Physiological plasticity enables *Cordylophora* to survive fluctuations in salinity and is a principle feature enabling the invasion of nonnative waters (Ellis & MacIsaac 2009; Leuven et al. 2009; Kestrup & Ricciardi 2010). As aquatic ecosystems are exposed to salinization due to natural and anthropogenic factors, it is important to know how aquatic invertebrates, especially invasive species such as *Cordylophora,* are able to tolerate and acclimate to changes in salinity

(Zalizniak et al. 2009). Salinization has been suggested to be an important factor in favoring the establishment of this invasive hydroid and other cnidarians in novel habitats (Ma & Purcell 2005; Purcell et al. 2007), and since hydranth production in the freshwater clade 1A genotype was optimal at 0.5–2 psu, partial salinization of freshwater habitats will likely facilitate the presence of this hydroid. Salinity levels in estuarine habitats such as our collection site in the Napa River in California may have fluctuated more recently due to the increased demand for freshwater because of the California drought (T. Giovannoni, unpubl. data). The reduced flow of the river promoted the movement of a dense saltwater wedge upriver potentially altering the historical distribution of organisms in these habitats. Future studies need to address distribution patterns in conjunction with ecophysiological features of this invasive euryhaline hydroid.

Conclusion

This study has reported the characteristic growth rates and hydranth morphologies for two genotypes of *Cordylophora* at a wide range of salinities. Our growth and morphological data from both genotypes suggest that there are indeed differences in growth rates, suggesting distinct optimal salinity ranges. There are also differences in hydranth morphology between the freshwater and brackish genotypes, and hydranth shape changes when grown at salinities above or below the native environment. These growth and morphology results can possibly be explained by mechanisms of osmoregulation in other freshwater and brackish cnidarians. This study is the first to assess growth and morphology in a freshwater lineage of *Cordylophora*, and we have demonstrated distinct phenotypic differences between representatives of clades 1A and 2B that support the previously proposed existence of multiple cryptic species within the genus. It will be informative for future studies to investigate multiple genotypes from the other two clades (2A and 1B), especially 1B, which includes colonies from freshwater and brackish habitats. In addition, our results raise several important questions for further inquiry. What are the specific physiological responses to osmotic stress in *Cordylophora*, and what are the processes allowing it to survive such a wide range of salinities? And what are the mechanisms behind hydranth morphological plasticity? *Cordylophora* will no doubt serve as an intriguing model for future research into cnidarian ecophysiology.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

Amado EM, Vidolin D, Freire CA, & Souza MM 2011. Distinct patterns of water and osmolyte control between intertidal (*Bunodosoma caissarum*) and subtidal (*Anemonia sargassensis*) sea anemones. Comp. Biochem. Physiol. Part A 158: 542–555.

Arndt EA 1984. The ecological niche of *Cordylophora caspia* (Pallas, 1771). Limnologica. 15(2): 469–477.

Benos DJ & Prusch RD 1972. Osmoregulation in freshwater *Hydra*. Comp. Biochem. Physiol. 43A: 165–171.

Benos DJ & Prusch RD 1973. Osmoregulation in *Hydra*: column contraction as a function of external osmolality. Comp. Biochem. Physiol. 44A: 1397–1400.

Benos DJ, Kirk RG, Barba WP, & Goldner MM 1977. Hyposmotic fluid formation in *Hydra*. Tissue Cell 1: 11–22.

Benson-Rodenbough B & Ellington WR 1982. Responses of the euryhaline sea anemone *Bunodosoma cavernata* (BOSC) (Anthozoa, Actinaria, Actiniidae) to osmotic stress. Comp. Biochem. Physiol. 72A: 731–735.

Berezina NA & Panov VE 2004. Distribution, population structure and salinity tolerance of the invasive amphipod *Gmelinoides fasciatus* (Stebbing) in the Neva Estuary (Gulf of Finland, Baltic Sea). In: Biology of the Baltic Sea, Kautsky H & Snoeijs P, eds., pp. 199–206. Kluwer Academic Publishers, Netherlands.

Blezard DJ 1999. Salinity as a refuge from predation in a nudibranch-hydroid relationship within the Great Bay estuary system. M.S. thesis, University of New Hampshire, New Hampshire. 67 pp.

Carlton JT 2009. Deep invasion ecology and the assembly of communities in historical time. In: Biological Invasions in Marine Ecosystems. Ecological Studies. No. 204. Rilov G & Crooks JA, eds., pp. 13–56. Springer-Verlag, Berlin.

Chester CM, Turner R, Carle M, & Harris LG 2000. Life history of a hydroid/nudibranch association: a discrete-event simulation. Veliger. 43(4): 338–348.

Conde A, Domínguez JM, Novais J, & Ramil F 2013. First record of *Cordylophora caspia* (Hydrozoa: Cnidaria) in the Tagus estuary, central Portugal. Mar. Biodivers. Rec. 6: 106.

Deaton LE & Hoffmann RJ 1988. Hypoosmotic volume regulation in the sea anemone *Metridium senile*. Comp. Biochem. Physiol. 91C(1): 187–191.

Dobrzycka-Krahel A & Graca B 2014. Laboratory study of the effect of salinity and ionic composition of water on the mortality and osmoregulation of the gammarid amphipod *Dikerogammarus haemobaphes* (Eichwald, 1841): implications for understanding its invasive distribution pattern. Mar. Freshw. Behav. Physiol. 47(4): 227–238.

Ellis S & MacIsaac HJ 2009. Salinity tolerance of Great Lakes invaders. Freshwater Biol. 54: 77–89.

Fleming WR & Hazelwood DH 1967. Ionic and osmoregulation in the fresh-water medusa, *Craspedacusta sowerbyi*. Comp. Biochem. Physiol. 23: 911–915.

Folino-Rorem NC 2015. Phylum Cnidaria. In: Ecology and General Biology: Thorp and Covich's Freshwater Invertebrates, Thorp J & Rogers DC, eds., pp. 159–179. Academic Press, Amsterdam.

Folino-Rorem NC, Darling JA, & D'Ausilio CA 2009. Genetic analysis reveals multiple cryptic invasive species of the hydrozoan genus *Cordylophora*. Biol. Invas. 11: 1869–1882.

Fraser CM 1944. Hydroids of the Atlantic coast of North America. The University of Toronto Press, Toronto. 315 pp.

Fulton C 1962. Environmental factors influencing the growth of *Cordylophora*. J. Exp. Zool. 151: 61–78.

Gilles R 1979. Intracellular organic osmotic effectors. In: Mechanisms of Osmoregulation in Animals: Maintenance of Cell Volume. Gilles R, ed., pp. 111–154. Wiley-Interscience Publication.

——— 1987. Volume Regulation in Cells of Euryhaline Invertebrates. Curr. Top. Membr. Transp. 30: 205–247.

Govindarajan AF & Carman MR 2016. Possible cryptic invasion of the Western Pacific toxic population of the hydromedusa *Gonionemus vertens* (Cnidaria: Hydrozoa) in the Northwestern Atlantic Ocean. Biol. Invas. 18: 463–469.

Grabowski M, Bacela K, Konopacka A, & Jazdzewski K 2009. Salinity-related distribution of alien amphipods in rivers provides refugia for native species. Biol. Invas. 11: 2107–2117.

Hazelwood DH, Potts WTW, & Fleming WR 1970. Further studies on the sodium and water metabolism of the fresh-water medusa, *Craspedacusta sowerbyi*. J. Comp. Physiol. 67(2): 186–191.

Hoffmann EK, Lambert IH, & Pedersen SF 2009. Physiology of cell volume regulation in vertebrates. Physiol. Rev. 89(1): 193–277.

Jankowski T, Collins AG, & Campbell R 2008. Global diversity of inland cnidarians. Hydrobiologia 595: 35–40.

Kalaus M, & Ojaveer H 2014. Over one decade of invasion: the non-indigenous cladoceran *Evadne anonyx* G.O. Sars, in a low-salinity environment, 1897. Aquat. Invas. 9(4): 499–506.

Kasschau MR, Ragland JB, Pinkerton SO, & Chit EC 1984. Time related changes in the free amino acid pool of the sea anemone, *Bunodosoma cavernata*, during salinity stress. Comp. Biochem. Physiol. 79A(1): 155–159.

Kaushal SS, Groffman PM, Likens GE, Belt KT, Stack WP, Kelly VR, Band LE, & Fisher GT 2005. Increased salinization of fresh water in the northeastern United States. Proc. Natl. Acad. Sci. 102: 13517–13520.

Kefford BJ, Buchwalter D, Cañedo-Argüelles M, Davis J, Duncan RP, Hoffmann A, & Thompson R 2016. Salinized rivers: degraded systems or new habitats for salt-tolerant faunas? Biol. Lett. 12(3): 20151072.

Kestrup A & Ricciardi A 2010. Influence of conductivity on life history traits of exotic and native amphipods in the St. Lawrence River. Fundam. Appl. Limnol. Arch. Hydrobiol. 176(3): 249–262.

Khlebovich VV 1990. Some physio-chemical and biological phenomena in the salinity gradient. Limnologica 20 (1): 5–8.

Kinne O 1956. Über den Einfluß des salzgehaltes und der temperature auf wachstum, form, und vermehrung bei dem hydroidpolypen *Cordylophora caspia* (Pallas), Athecata. Clavidae. Zool. Jb. (Physiol.) 66: 565–638.

——— 1958. Adaptation to salinity variations–some facts and problems. In: Physiological Adaptation. Prosser CL, ed., pp. 92–106. American Physiological Society, Washington, DC.

——— 1964. The effects of temperature and salinity on marine and brackish water animals. II. salinity and temperature salinity combinations. Oceanogr. Mar. Biol. Ann. Rev. 2: 281–339.

——— 1971. Salinity: invertebrates. In: Marine Ecology, Kinne O, ed., pp. 821–995. Wiley-Interscience, New York.

Kinne O & Paffenhöfer G 1965. Hydranth structure and digestion rate as a function of temperature and salinity in *Clava multicornis* (Cnidaria, Hydrozoa). Helgoländer Wiss. Meeresunters 12: 329–341.

——— 1966. Growth and reproduction as a function of temperature and salinity in *Clava multicornis* (Cnidaria, Hydrozoa). Helgoländer Wiss. Meeresunters 13: 62–72.

Lange R 1972. Some recent work on osmotic, ionic and volume regulation in marine animals. Oceanogr. Mar. Biol. Ann. Rev. 10: 97–136.

Leppäkoski E, Gollasch S, Gruska P, Ojaveer H, Olenin S, & Panov V 2002. The Baltic—a sea of invaders. Can. J. Fish Aquat. Sci. 59: 1175–1188.

Leuven RSEW, van der Velde G, Baijens I, Snijders J, van der Zwart C, Lenfers HJR, & bij de Vaate A 2009.

The river Rhine: a global highway for dispersal of aquatic invasive species. Biol. Invas. 11: 1989–2008.
Lilly SJ 1955. Osmoregulation and ionic regulation in *Hydra*. J. Exp. Biol. 32: 423–439.
Ma X & Purcell JE 2005. Effects of temperature, salinity, and predators on mortality of and colonization by the invasive hydrozoan *Moersia lyonsi*. Mar. Biol. 147: 215–224.
Meek MH, Wintzer AP, Wetzel WC, & May B 2012. Climate change likely to facilitate the invasion of the nonnative hydroid, *Cordylophora caspia*, in the San Francisco Estuary. PLoS ONE 7(10): e46373. https://doi.org/10.1371/journal.pone.0046373.
Morabito R, Marino A, Peter K, & La Spada G 2013. Sea water acidification affects osmotic swelling, regulatory volume decrease and discharge in nematocytes of the jellyfish *Pelagia noctiluca* cell. Physiol. Biochem. Part A 165: 199–206.
Muskó IB, Bence M, & Balogh CS 2008. Occurence of a new Ponto-Caspian invasive species, *Cordylophora caspia* (Pallas 1771) (Hydrozoa: Clavidae) in Lake Balaton (Hungary). Acta. Zool. Acad. Sci. Hung. 54(2): 169–179.
Obolewski K, Jarosiewicz A, & Ozgo MI 2015. Invasive Ponto-Caspian hydrozoan *Cordylophora caspia* (Hydrozoa: Cnidaria) in southern Baltic coastal lakes. Estuar. Coast. Shelf Sci. 167: 147–153.
Ordóñez V, Pascual M, Fernández-Tejedor M, & Turon X 2016. When invasion biology meets taxonomy: *Clavelina oblonga* (Ascidiacea) is an old invader in the Mediterranean Sea. Biol. Invas. 18: 1203–1215.
Paavola M, Olenin S, & Leppäkoski E 2005. Are invasive species most successful in habitats of low native species richness across European brackish water seas? Estuar. Coast. Shelf Sci. 64: 738–750.
Pierce SK 1982. Invertebrate cell volume control mechanisms: a coordinated use of intracellular amino acids and inorganic ions as osmotic solute. Biol. Bull. 163(3): 405–419.
Prusch RD, Benos DJ, & Ritter M 1976. Osmoregulatory control mechanisms in freshwater Coelenterates. Comp. Biochem. Physiol. 53(2): 161–164.
Purcell JE, Uye S, & Lo W 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. Mar. Ecol. Prog. Ser. 350: 153–174.
Ricciardi A 2015. Ecology of invasive alien invertebrates. In: Ecology and General Biology: Thorp and Covich's Freshwater Invertebrates. Thorp J & Rogers DC, eds., pp. 83–91. Academic Press, Amsterdam.
Schuchert P 2004. Revision of the European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Families Oceanidae and Pachycordylidae. Rev. Suisse Zool. 111(2): 315–369.
Sokołowski A, Brulin′ska D, Olenycz M, & Wołowicz M 2016. Does temperature and salinity limit asexual reproduction of *Aurelia aurita* polyps (Cnidaria: Scyphozoa) in the Gulf of Gdan′sk (southern Baltic Sea)? An experimental study. Hydrobiologia 773: 49–62.
Stebbing ARD 1981. The effects of reduced salinity on colonial growth and membership in a hydroid. J. Exp. Mar. Biol. Ecol. 55: 233–241.
Steinbach HB 1963. Sodium, potassium and chloride in selected hydroids. Biol. Bull. 124(3): 322–336.
bij de Vaate A, Jazdzewski K, Ketelaars HAM, Gollasch S, & van der Velde G 2002. Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. Canadian Journal of Fisheries and Aquat. Sci. 59: 1159–1174.
del Valle JC, Acuña FH, & Manañes AAL 2015. Digestive flexibility in response to environmental salinity and temperature in the non-symbiotic sea anemone *Bunodosoma zamponii*. Hydrobiologia 759: 189–199.
Webb KL, Schimpf AL, & Olmon J 1972. Free amino acid composition of scyphozoan polyps of *Aurelia aurita*, *Chrysaora quinquecirrha* and *Cyanea capillata* at various salinities. Comp. Biochem. Physiol. 43B(3): 653–663.
Williams WD 2001. Anthropogenic salinization of inland waters. Hydrobiologia 446: 329–337.
Wintzer AP, Mariah H, Meek MH, Peter B, Moyle PB, & May B 2011. Ecological insights into the polyp stage of non-native hydrozoans in the San Francisco Estuary. Aquat. Ecol. 45: 151–161.
Zalizniak L, Kefford BJ, & Nugegoda D 2009. Effects of pH on salinity tolerance of selected freshwater invertebrates. Aquat. Ecol. 43: 135–144.